# Trace Analysis

WHERE very small quantities of materials are determined or detected, we soon find that we are faced with unique problems. .The samples used are generally quite large relative to the amount of material being determined, and this presents problems of separating the wanted from the unwanted. There can be, of course, definite difficulties in sampling if uniformity is absent. ' Furthermore, the elements or compounds sampled are most often in concentrations of parts per million or parts per billion, and they may be of known composition, or the traces may have to be identified before a method can be developed for their quantitative determination. Trace materials and analytical methods for their study are rather taken for granted in catalytic, nutrition, and biochemical research. Air pollution studies until recently have been largely educated guesses as to what materials and concentrations were causing the pollution. Stream pollution studies are about at the same stage of development.

The programs for air pollution research have been centered largely around analytical research to perfect methods for rapid surveys to better define the problems and so determine what measures can be taken to correct conditions. Air pollution has been the subject of much discussion in the lay press and in scientific circles, and because the air we breathe is the concern of everyone of us, the laws passed and the money spent can be subject to much unscientific bias. Air pollution can be caused by liquids, gases, and solids as such, or in various combinations, so that the problem is exceedingly complex. Odor may be present and may be from a harmful or harmless compound. Lack of odor is not a sign of healthful air.

It is to the credit of the various groups concerned with these studies that analytical tools are recognized to be of prime importance in collecting data from which scientific conclusions can be drawn and later legislation enacted. It will be the responsibility of the analyst to monitor conditions to see that laws are not broken.

It is also encouraging to see that many instruments are of the recording type so that 24-hour records of contaminants can be carried out. Here infrared, ultraviolet, mass spectrometry, and the automatic counting of dust particles are all used in the analytical program.

In both stream and air pollution the biochemist and biologist play a leading role in their studies of the effect of trace materials on life. .The public naturally clamors for action, but it is better to spend the millions which will be spent on control by industry for those conditions which are actually causing health hazards.

# Chronopotentiometric Titrations

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A new method of end-point detection based upon chronopotentiometry is described. The relationship between chronopotentiometric and potentiometric titrimetry, the possible types of titration curves, and the factors which govern the sensitivity of the method are discussed. Although chronopotentiograms of a permanent sort are obtained more easily with a recorder, variable transition times for titrations can be measured simply and conveniently with a B battery, variable resistor, pH meter, and stop watch. The method is illustrated by the titration of ferric ion with ceric ion, copper ion with (ethylenedinitrilo)tetraacetic acid, arsenite ion with iodine, and the successive titration of a mixture of stannous and ferrous ions with ceric ion. The method has the advantages of the amperometric method, but is applicable to the titration of small volumes or concentrations, and in situations where stirring is undesirable.

IN RECENT years an increasing number of new titration procedures have been developed. The coulometric method of reagent addition and measurement is suitable for titration of extremely minute amounts of materials. However, end-point procedures which allow detection of a given species in this region are lacking, and full advantage cannot be taken of the coulometric method. The advent of new titrants, such as the complexons, and recognition of the importance of the solvent, such as in the Karl Fischer method and in nonaqueous acid-base titrations, again have focused attention on end-point procedures that would be especially suitable. Because each new titration procedure presents its own problem of end-point detection, studies of new techniques, such as reported in this paper, may be especially useful.

## PRINCIPLE

The theory of chronopotentiometry has been reviewed by Delahay and Mamantov (1), and the experimental aspects have been described by Reilley, Everett, and Johns (3). In the chronopotentiometric method the solution is allowed to come to a complete state of rest, then a constant current is applied suddenly across an electrode-surface interface, and the resulting transient potential variation occurring at the interface is followed as a function of time (thus chronopotentiometry).

Because the solution contains a supporting electrolyte and because the electrode surface is usually flat, the mode of transfer of the active species to the surface is controlled by linear diffusion. Under these conditions the interface potential is found upon application of current to progress rapidly to the decomposition potential of the species present. After a certain length of time (the transition time) the concentration of that species at the interface is depleted entirely, and the potential then progresses rapidly to the decomposition potential of some other species or the solvent.

One of the fundamental equations of chronopotentiometry, derived by Sand (4),

$$C = \frac{2i\tau^{1/2}}{nFA\,D^{1/2}\pi^{1/2}} \tag{1}$$

states that the concentration, $C$ (moles cm.$^{-2}$), of a single species being electrolyzed under linear diffusion control conditions at a constant current, $i$ (ampere), is directly proportional to the square root of the transition time, $\tau$ (second). $A$ is the area (sq. cm.) of the electrode, $n$ is the number of electrons involved in the elec-

trolysis for each molecule or ion, $F$ is the faraday (ampere second), $\pi$ is 3.142, and $D$ is the diffusion coefficient (sq. cm. sec.$^{-1}$).

Therefore, if chronopotentiograms for a species involved in the titration reaction are taken at a particular current density for several points during the titration, a plot of $\tau^{1/2}$ as a function of the volume of titrant yields two straight lines whose intersection corresponds to the end point. One of these lines may be the base line corresponding to complete depletion of the species where $\tau = 0$.

## SHAPE OF TITRATION CURVES

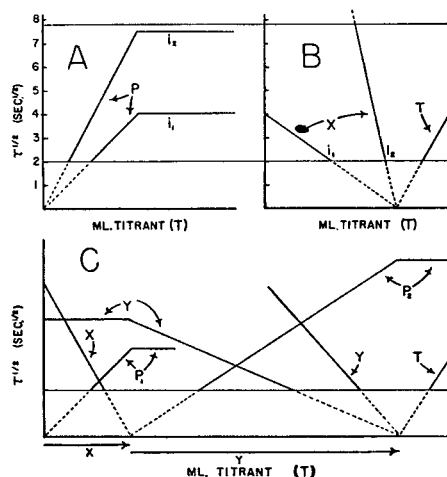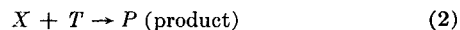**One Component.** Consider the general titration reaction:

$$X + T \rightarrow P \text{ (product)} \tag{2}$$



**Figure 1. General shape of titration curves**

A. Concentration of products $P$ followed chronopotentiometrically

B. Concentration of species titrated $X$ and titrant $T$ followed chronopotentiometrically

C. Successive titration of two-component mixture (Equations 3 and 4)

A chronopotentiometric end point can be obtained if unambiguous chronopotentiograms can be obtained for the substance titrated, $X$, the titrant, $T$, and/or the soluble products, $P$, as shown in Figure 1.

In general, transition times less than 4 seconds or greater than 60 seconds were avoided. The small transition times are difficult to measure accurately because of the time lag in the pH meter and recorder. Instrumentation for accurate measurement of smaller time intervals undoubtedly could be constructed but surface roughness and double layer capacitance have increasing influence under these conditions (3) and deviations from Equation 1 occur at the small concentrations present in the vicinity of the end point. Transition times longer than 60 seconds are avoided because the density of the solution usually varies across the diffusion layer, and this variation eventually leads to convection. The transfer of the active species to the electrode surface therefore is not controlled solely by thermal diffusion and deviations from Equation 1 become large and erratic.

Figure 1,A, illustrates the shape of titration curves expected when the concentration of the soluble products is followed at two values of current, $i_2$ and $i_1$, where $i_2$ is less than $i_1$. The end point

corresponds to the titrant volume where further addition of the titrant gives no further increase in the product concentration.

The error in determining the exact point of intersection decreases as the difference in the slope for the two lines increases. For this reason $i_2$ is a better choice of current than $i_1$. Unless the product reacts further with the titrant, the slope of the line beyond the end point is zero. Therefore, the slope of the line prior to the end point should be as large as possible; thus the difference between the transition times at the beginning of the titration and at the end point should be large. The transition time at the beginning of the titration is usually zero (unless some product is present in the sample), and the effectiveness of the titration is governed solely by the transition time at the end point, which has a practical maximum value of about 60 seconds. Because of these inherent limitations in decreasing the relative error, a titration where the concentration of a product is followed is avoided whenever possible.

The end point may also be determined by noting the decrease in concentration of the substance titrated, $X$, or by observing the increase in concentration of the titrant, $T$ (Figure 1,$B$). There are two slopes shown for $X$, illustrating the effect of current magnitude on transition times. With current $i_1$ no points are obtained for the line beyond 3 ml. of titrant, but the approximate end point can be predicted. However, it is possible to employ a lower current $i_2$ and to obtain points closer to the end point (about 4 to 5 ml.). The relative error in determining the end point therefore is decreased appreciably by the use of small currents such as $i_2$.

Theoretically, the end point does not correspond to the intersection of line $X$ with the zero axis, but rather to the intersection of line $X$ with a line obtained in the absence of $X$. However, since this line generally lies very nearly on the zero axis, this correction may be eliminated. In the titration of very dilute solutions, this correction is more important.
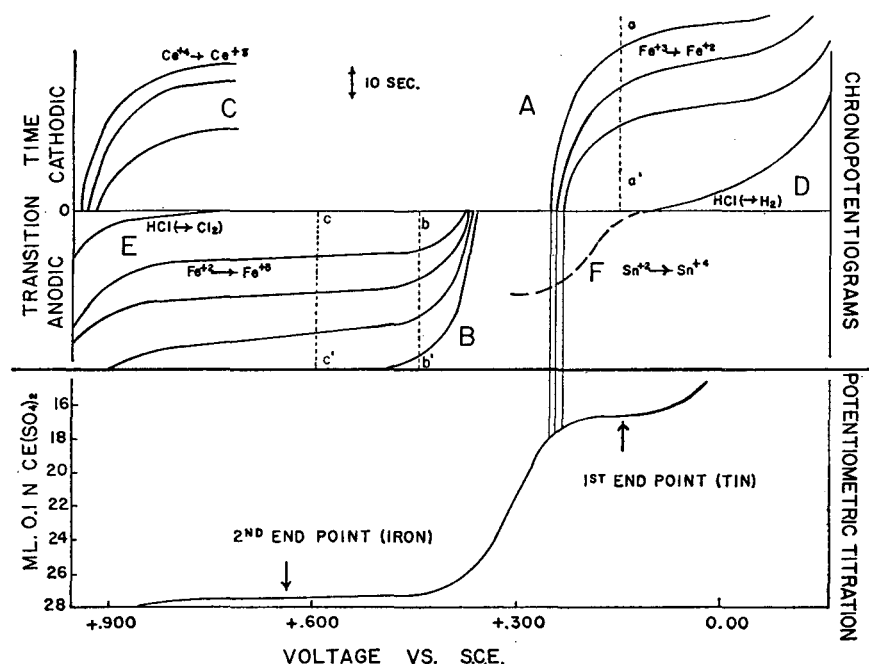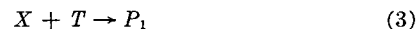
In a similar manner, the increase in concentration of the titrant may be used to determine the end point, especially in cases where the reactant is not electroactive or does not yield well defined chronopotentiograms—i.e., arsenite ion titrated with iodine.

**Two Components.** Consider the consecutive reaction:

$$X + T \rightarrow P_1 \tag{3}$$

followed by

$$Y + T \rightarrow P_2 \tag{4}$$

In the titration of a two-component mixture, measurements may be made on the change in concentration of any one of the components ($X$, $Y$), their products ($P_1$, $P_2$), and/or the titrant ($T$). These various possibilities are illustrated in Figure 1,$C$. The end point for the titration of component $X$ may be detected best by following the decreasing concentration of component $X$ prior to the end point, or the sudden increase in product $P_2$ after the first end point. Component $Y$ or product $P_1$ also can be followed but with greater error in determining the end point. In the analysis of a single-component system, a second component analogous to $Y$ can be introduced to serve as an indicator (preferably by detecting appearance of its product, $P_2$) in cases where $X$ or $T$ does not yield a satisfactory chronopotentiogram, thus avoiding the procedure illustrated in Figure 1,$A$.

The second end point corresponding to the titration of component $Y$ may be detected by following the decreasing concentration of $Y$ (the use of lower current density allowing a steeper line as shown), the sudden increase in concentration of titrant $T$, or less satisfactorily, the concentration of product $P_2$. However, both end points can be detected by making measurements on one electroactive species only, either $Y$ or $P_2$.

Single chronopotentiograms may not be obtainable for each of these species, practically and theoretically. In the oxidation-reduction titration of a mixture of ceric and ferric ions with a reducing agent, the reduction theoretically and experimentally exhibits two steps— the first representing the transition time for the reduction of ceric ions to cerous ions, and the second corresponding to the reduction of ferric ions to ferrous ions. In chronopotentiometry, the presence of the first step alters the height of the second step ($1$, $3$). Although a plot of $(\tau_1 + \tau_2)^{1/2}$ versus milliliters yields straight-line titration curves, this situation must be considered in establishing the best procedure.

**Sensitivity Factors.** The error in locating the end point is related closely to the slope of the titration line (Figure 1). Also, the lower limit of concentration for the chronopotentiometric method depends strongly on the steepness of the slope which can be obtained.

The slope is essentially proportional to the variation of $\tau^{1/2}$ for a given variation in the milliequivalents (meq.) of the species being detected:

$$\text{Slope} = \frac{d\tau^{1/2}}{d \text{ meq.}} = \frac{nF\pi^{1/2}D^{1/2}A}{2iV} \tag{5}$$

For a given species, $n$, $F$, and $D$ are



**Figure 2. Relationship of chronopotentiometric titration (upper curves) to potentiometric titration (lower curve)**

Sn$^{+2}$ + 2Ce$^{+4}$ → 2Ce$^{+3}$ + Sn$^{+4}$ (first reaction)
Fe$^{+2}$ + Ce$^{+4}$ → Ce$^{+3}$ + Fe$^{+3}$ (second reaction)
Chronopotentiograms for
*A.* Ferric reduction slightly past first end point
*B.* Ferrous oxidation approaching second end point
*C.* Ceric reduction slightly past second end point
*D, E.* Supporting electrolyte, 6$N$ HCl
*F.* Stannous oxidation (in absence of ferrous)

constants and the experimentally variable factor controlling the sensitivity becomes:

$$\text{Sensitivity factor} = \frac{A}{iV} \qquad (6)$$

Thus for a given amount of material to be titrated, a greater sensitivity is obtained for larger electrode area, $A$, smaller current, $i$, and smaller volume of solution, $V$.

Equation 6 shows that for any given electrode area and solution volume, the desired sensitivity may be obtained by adjusting the current value employed. This is true only within certain limits of $A$ and $V$.

First the selection of electrode area may be considered. A very large area may result in serious depletion by way of electrolysis and low results will be obtained. Also, higher currents than are available may be necessary to remain within the 60-second limit for transition times. On the other hand, electrodes which are too small are more susceptible to nonlinear diffusion (fringe effects) and to convection currents. The relative effect of the double layer capacitance is independent of electrode size.
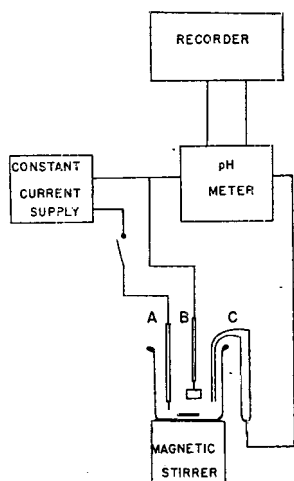


Figure 3. Schematic circuit for chronopotentiometric titration

A second consideration is the volume of solution. Dilution corrections are necessary if the initial volume is too small relative to the titrant volume. Small volumes have a decided advantage in decreasing the relative effect of the double layer capacitance, an effect which becomes troublesome for solutions below $10^{-5}M$.

In practice, the electrode area and volume are selected first, and then a current is selected to give the desired sensitivity factor.

### RELATION TO POTENTIOMETRIC TITRATION

The relationship between chronopotentiometric titrations and potentiometric titrations is shown in Figure 2. The data presented are for an actual titration of a mixture of stannous ions and ferrous ions with $0.10N$ ceric sulfate. The lower part of the figure represents the potentiometric titration with the voltage plotted as the abscissa and volume of titrant as the ordinant. The upper part of the figure illustrates chronopotentiograms taken during the course of the titration. The line, $D$—$E$, was obtained for the medium, $6N$ hydrochloric acid alone.

An anodic wave, $F$, was observed for the oxidation of stannous ion when it was the only oxidizable metal ion in solution. A large degree of overvoltage is noticeable. In the presence of ferrous ions, the two waves tended to merge. Therefore, the stannous ion end point was determined by observing the increase in concentration of the ferric ions, $A$, immediately after the first
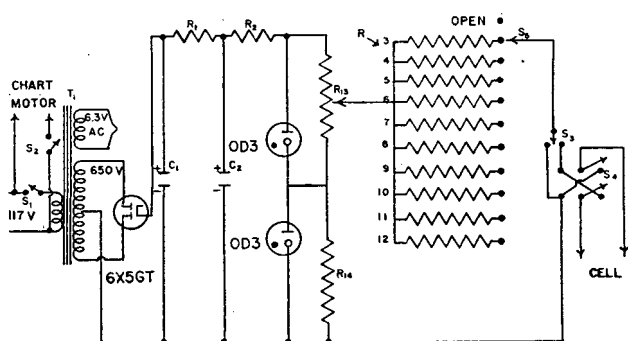


Figure 4.   Circuit diagram for constant current supply

$R_1$.    1000 ohms, 2-watt resistor
$R_2$.    4000 ohms, 2-watt resistor
$R_3$.    32 megohms, 1-watt resistor
$R_4$.    16 megohms, 1-watt resistor
$R_5$.    8 megohms, 1-watt resistor
$R_6$.    4 megohms, 1-watt resistor
$R_7$.    2 megohms, 1-watt wire-wound resistor
$R_8$.    1 megohm, 1-watt wire-wound resistor
$R_9$.    500,000 ohms, 1-watt wire-wound resistor
$R_{10}$.   200,000 ohms, 1-watt wire-wound resistor
$R_{11}$.   100,000 ohms, 1-watt wire-wound resistor
$R_{12}$.   50,000 ohms, 2-watt wire-wound resistor
$R_{13}$.   20,000 ohms, 10-turn wire-wound Helipot (Beckman Instrument Co.)
$R_{14}$.   20,000 ohms, 2-watt resistor
$C_1$, $C_2$.    8 mfd. electrolytic condenser, 450 volts
$T_1$.    650 V.C.T., about 55 ma., 6.3 volts, about 2 amp. (Stancor PC-8406)
$S_1$, $S_2$.    SPST switch
$S_3$.    SPDT three-position switch
$S_4$.    DPDT two-position switch
$S_5$.    11-position switch (Mallory 181 C)
Chassis.   7 × 7 × 2 inches

end point. No separate wave for the reduction of stannic ion was observed.

The ferrous end point was determined by noting its decrease in concentration, $B$, just prior to the end point or by observing the increase in concentration of ceric ion, $C$, immediately past the end point. No anodic wave for the oxidation of cerous ion was observed.

The initial voltage (zero time) of the chronopotentiogram corresponds to the potentiometric voltage (shown by faint connecting lines between the potentiometric curve and chronopotentiograms, $A$). By observing the change in voltage as the titration proceeds, the points can be predicted at which chronopotentiograms should be taken. Whether the end point has been exceeded is determined potentiometrically.

The proper selection of voltage at which the transition times are measured depends upon the shape of the chronopotentiogram. Any point, past the holdup voltage for the species being detected, theoretically may be used, provided this same point is used for all measurements. It is usually desirable to make measurements in a region where the chronopotentiogram is fairly flat and horizontal. However, this condition is not always realized because of the influence of the double layer capacitance, and it may be necessary to make measurements on a sloping portion just past the major potential holdup (Figure 2, lines $a$ $a'$, $c$ $c'$). Line $b$ $b'$ illustrates a poor choice of voltage because the level portion of the chronopotentiogram has not yet been reached.

### APPARATUS

Transition times may be determined most simply with a B battery, variable resistor, voltmeter, and stopwatch. However, for the purpose of checking the method, a recorder is used in the measuring circuit to obtain a record of the chronopotentiograms. In addition, a calibrated electronic current supply is used.

**Circuit.** The arrangement of the apparatus used is shown in Figure 3. The current is passed through the two platinum electrodes as shown. Then the chronopotentiograms are observed by measuring the voltage change between one platinum electrode and a saturated calomel reference electrode as a function of time. The Leeds and Northrup pH indicator (Catalog No. 7664-A1) used as a direct-reading millivoltmeter is connected to a Leeds and Northrup Speedomax Type G recorder. This recorder has a chart speed of 480 inches per hour and a scanning time of 1 second.

**Current Supply.** A simple current supply shown in Figure 4 supplies any desired constant current from 5 μa. to 2.5 ma.

**Time Measurement.** An electrical timer (Dimco Gray Co., Dayton, Ohio, Series 202) is used in the experiments where transition times are measured by watching the movement of the milli-voltmeter needle.

### REAGENTS

**Titrants.** Approximately 0.10N solutions of ceric ammonium sulfate, iodine, and disodium salt of (ethylenedinitrilo)tetraacetic acid (dihydrogen disodium Versenate) (magnesium-free) are prepared in the usual manner.

**Reactants.** Approximately 0.10N solutions of copper sulfate, ferric chloride, sodium arsenite, and stannous chloride were prepared in the usual manner.

**Other.** Osmium tetroxide, 0.01M from G. F. Smith Chemical Co., Columbus, Ohio. Nitrogen, Airco Seaford grade, used without further purification. Potassium sulfate, 0.10N, used as a background electrolyte in the copper–(ethylenedinitrilo)tetra-acetic acid titration.

### PROCEDURE

In order to test the chronopotentiometric method of end-point detection, oxidation-reduction and complexometric titrations were carried out. The correctness of the method was checked against a potentiometric end point in the case of the redox titrations and against a conductometric end point in the case of the complexometric titration.

**Electrode Size.** In cases where small concentrations were titrated, the platinum foil (2 sq. cm.) was used as the indicator

electrode. A platinum wire (26 gage, 1 cm. long) electrode can be used for titrations involving large concentrations.

**Current Values and Transition Times.** Concentration measurements—i.e., transition times—are made at any point during the titration. However, measurements are made best near the end point because of the steeper slopes obtained. At some suitable point in the titration, the stirrer is stopped and the solution allowed to become quiet. The selection of the current magnitude and the transition time depends upon the shape of the titration curve expected. To measure the decrease in concentration of an ion (Figure 1,B), the initial transition time should not exceed 60 seconds. The current is adjusted until approximately this value is obtained. If the concentration of an ion is measured which is small at the outset and large at the final measurement (Figure 1,A), then a current value is selected so that the initial transition time is about 4 to 5 seconds.
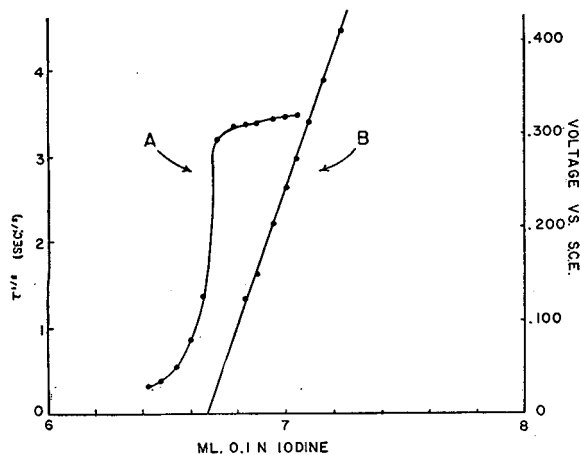
**Figure 7. Titrations of ferrous ion with ceric ion**

Chronopotentiometric titrations
A. Recorder method: i is 138 μa.; end point is 27.69 ml.
B. Recorder method: i is 76.6 μa.; end point 27.69 ml.
C. Visual method: i is 40.1 μa.; end point is 27.71 ml.
D. Potentiometric, end point 27.70 ml.

**Titration.** Once a suitable current has been selected, the titrant is added in small increments and three transition times for each increment are obtained. The end point is obtained by plotting the square root of the average transition time against the milliliters of titrant and extrapolating to zero time (for curves like Figure 1, B). If more than one titration line is desired, the current during the titration is lowered or raised accordingly.

The solution must be absolutely quiet when the transition time measurements are made. The cell was mounted on a stone-base ring stand, which was in turn placed on rubber mountings. The whole cell assembly was placed on one table, and the recorder, current supply, and pH meter were placed on a separate table. In this way, vibrations caused by the recorder and by the operation of the current supply switches were eliminated.

A constant time of 100 seconds was allowed to elapse between stopping the magnetic stirrer and starting the current.

### RESULTS

**Arsenite vs. Iodine** (Figure 5). A buffered solution of arsenite ion was titrated with iodine and the course of the titration was followed potentiometrically. After the end point was passed, the sudden increase in the concentration of the iodine was followed chronopotentiometrically. The results of the chronopotentiometric and potentiometric methods agree within 1.5 parts per thousand. The shortest transition time in Figure 5 (also Figure 9), corresponds to detecting the presence of $3 \times 10^{-5}M$ iodine. A platinum foil electrode, 2 sq. cm. in area, was used in the titration, and the transition times were measured at 0.100 volt vs. S.C.E.

**Copper vs. (Ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid)** (Figure 6). The procedure was checked by comparison with a conductometric titration (2). Five milliliters of 0.10M copper sulfate were diluted to 100 ml. with 0.10M potassium sulfate and the resulting solution was bubbled with nitrogen for 10 minutes. A mercury-plated platinum foil electrode (2 sq. cm.) prepared by first plating a thin film of copper on a freshly cleaned platinum foil at 50 μa. for a few seconds and then

**Figure 5. Titration of arsenite ion with iodine**

A. Potentiometric (end point at 6.68 ml.)
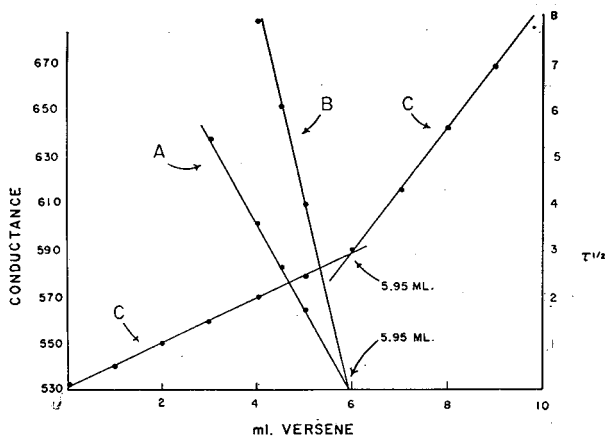B. Chronopotentiometric, end point at 6.67 ml.

**Figure 6. Titrations of copper ions with Versene**

A. Chronopotentiometric, i is 600 μa.
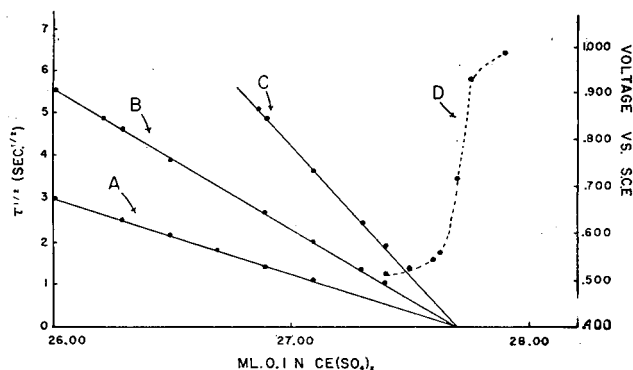B. Chronopotentiometric, i is 1.42 ma.
C. Conductometric

immersing in mercury, was used as the indicator cathode. Two different electrodes prepared in this way gave satisfactory results. The transition times were measured at −0.200 volt vs. S.C.E.

The course of the titration was followed by observing the decrease in concentration of the copper chronopotentiometrically. Slopes at two current values were obtained and compared with a conductometric titration of the same amount of copper. The conductometric titration was carried out in $1M$ ammonia.

**Ferrous vs. Ceric (Figure 7).** Twenty-five milliliters of stock ferrous chloride were diluted to 200 ml. with $0.10N$ sulfuric acid. The large platinum foil (2 sq. cm.) was used as the anode, and the decrease in concentration of ferrous ions was observed chronopotentiometrically. The increase in concentration of ferric ions could also have been observed using the foil electrode as a cathode.

This titration was utilized to compare the recorder and visual (manual) method for obtaining transition times. In the visual method a timer was started simultaneously with the current, and stopped when the pH meter read +0.700 volt vs. S.C.E. Both methods gave results agreeing within 0.01 ml. of the potentiometric end point.
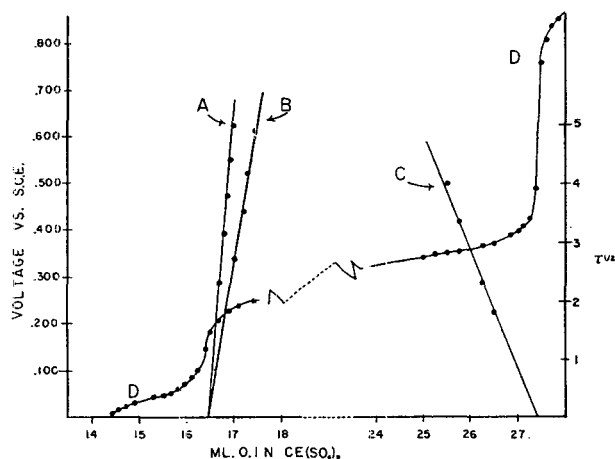


**Figure 8.  Titrations of stannous-ferrous mixture with ceric ion**

Chronopotentiometric titrations for
*A.*  Stannous end point;  *i* is 76.6 μa., end point is 16.41 ml.
*B.*  Stannous end point;  *i* is 40.1 μa., end point is 16.43 ml.
*C.*  Ferrous end point;  *i* is 145.5 μa., end point 27.39 ml.
*D.*  Potentiometric titration, end points at 16.40 and 27.41 ml.

**Stannous and Ferrous vs. Ceric (Figure 8).** The consecutive potentiometric titration of stannous and ferrous ions at 20° C. with ceric ions was attempted in several different acids of varying strength and with varying amounts of osmium tetroxide as a catalyst. The medium finally selected was $6N$ hydrochloric acid, with 3 drops of $0.01M$ osmium tetroxide. In other cases, none or poor potential breaks were observed in the vicinity of the expected stannous end point. Under the selected conditions, the titrant was added dropwise and a wait of a few minutes after each addition permitted equilibrium to be attained.

Two titration lines were obtained for the stannous end point by observing at two current values the increase in concentration of ferric ions past the end point as shown in Figure 8. The ferrous end point was determined by noting the decrease in ferrous ion concentration chronopotentiometrically. The 2-sq. cm. platinum foil was used as the cathode to determine the ferric ion concentration after the first end point and as an anode to determine the decrease of the ferrous ion concentration prior to the second end point. Transition times were measured at +0.150 volt vs. S.C.E. for the reduction of ferric ion and at +0.600 volt vs. S.C.E. for the oxidation of ferrous ion (see Figure 2).

**Typical Chronopotentiograms.** Typical chronopotentiograms

for ferric, ferrous, stannous, and ceric ions are illustrated in Figure 2. Figure 9 illustrates typical chronopotentiograms for the cathodic behavior of iodine and copper ion. The zero-time potential of the iodine curves shows the increase in potential as the concentration of iodine is increased. The iodine chronopotentiograms were obtained at a current of 19.8 μa. using the platinum foil electrode.

The magnitude of the transition time as a function of the square of the concentration is illustrated in the copper chronopotentiograms. The height of wave $C$ is clearly four times that of wave $A$, and similarly, the height of wave $D$ is four times that of wave $B$.
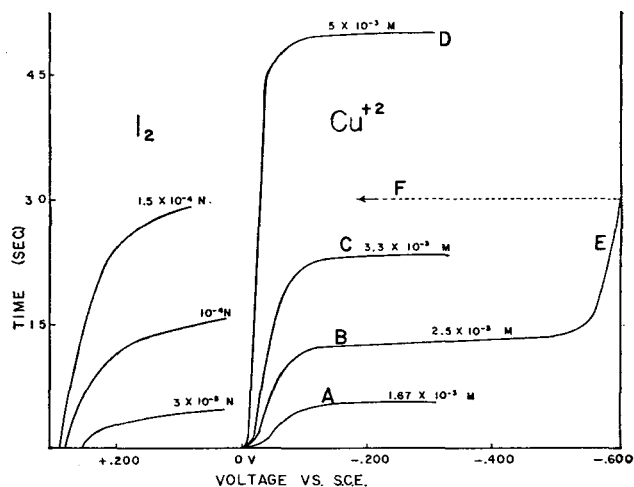


**Figure 9.  Typical chronopotentiograms**

*Left.*  Iodine to iodide
*Right.*  *A, B, C, D.*  Copper ion to copper (Hg)
*E.*  Beginning of cathodic wave for copper ethylene(dinitrilo)tetra acetate to copper(Hg)
*F.*  Anodic wave for copper(Hg) to copper ion, indicating slow reaction between copper ion and ethylene(dinitrilo)tetraacetic acid

The wave $E$ obtained upon continued electrolysis of a copper-(ethylenedinitrilo)tetraacetic acid mixture is attributed to the decomposition of the chelate. If the voltage scan had been allowed to continue, the wave height would have equaled approximately that of curve $D$. Because the process occurring at the electrode is reduction of the copper (ethylenedinitrilo)-tetraacetate to copper, ethylene(dinitrilo)tetraacetic acid is liberated in the vicinity of the electrode. Curve $F$ results upon reversing the polarity of the generating electrode and then copper ion is electrolyzed anodically into the solution from the copper-mercury amalgam. If the free (ethylenedinitrilo)-tetraacetic acid now present had recombined rapidly with the copper ion, an anodic wave would have resulted. The absence of such a wave indicates a slow reaction between copper ion and ethylene(dinitrilo)tetraacetic acid.

### CONCLUSION

The advantages of the method are allied closely with those of the amperometric method, and the basic principle of both is similar. The method can be applied to any titration system involving an electroactive constituent and the reactant titrant or product does not need to undergo a reversible electrode reaction, so long as diffusion of the electroactive species is the controlling factor of transition time.

The apparatus essential to the method is simple, being only a convenient source of current, a pH meter, and a stop watch.

Chronopotentiograms obtained with a platinum electrode are more reproducible than polarograms obtained with a rotating platinum electrode. Therefore, the chronopotentiometric method would be useful in the titration of materials in lower concentration regions and in the analysis of small volumes where reproducible stirring is difficult.

Under certain conditions, use of two platinum electrodes is possible where one acts essentially as a reference electrode, thus facilitating titrations where the usual reference electrodes are not desirable—e.g., in nonaqueous media.

The disadvantages are similar to those of amperometric titrations. Titrations in the negative potential region must be performed in the absence of oxygen, with a wait between additions of titrant during the measurements, and the end point requires a graphing of the results.

Precipitation titrations could not be performed easily, for erratic results are obtained unless the electrode surface is renewed before each run.

The method is more sensitive than amperometry to minor convection and vibration of the solution. Titrations that require heating would necessitate more rigorous temperature control to eliminate convection current.

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# Analysis of Reaction Products by Isotope Dilution Procedure

## Examination of Acrylic Acid–Ethyl Alcohol Reaction Mixtures for Diethyl Ether Formation

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**Small amounts of peroxide-forming impurities affect the polymerization characteristics of many monomers. This paper describes an investigation of the acrylic acid–ethyl alcohol reaction product to determine the amount of by-product ethyl ether present. As no chemical tests are available for the quantitative detection of small amounts of ether in the presence of large amounts of ester, the isotope dilution procedure using Carbon-14-labeled diethyl ether as the tracer was used. A number of different catalysts were used and small but definite amounts of diethyl ether were found in the reaction mixtures. The method is highly sensitive as applied to this system.**

THE nature of by-products encountered during the esterification of alpha-beta unsaturated carboxylic acids is a matter of considerable concern when these esters are to be used in subsequent polymerizations. This is especially true of by-product ethers which, because of peroxide formation, are capable of introducing an uncontrolled factor into peroxide-catalyzed polymerization reactions involving these esters (*1, 5, 6, 8, 10-12, 15, 18, 20*).

A search of the literature brought to light no published report of ether formation during esterification procedures. Nevertheless, esterification reactions involving the use of alcohols in the presence of acid catalysts (*4, 18*) do provide ideal conditions for ether formation (*13, 14, 21*). Therefore, it seemed advisable to investigate the extent of ether formation in a typical esterification involving a polymerizable acid.

Many chemical tests for ethers are known (*16, 17*), but they fail to detect small amounts of these compounds in the presence of large amounts of esters. Physical methods of analysis (such as infrared) as applied in this laboratory failed to indicate the presence of ether in the reaction products studied in these experiments. This implies that such impurities, if produced at all, are present in very small quantities. In view of these facts, it was decided to investigate the reaction between acrylic acid and ethyl alcohol and to use the isotope dilution procedure with diethyl-1-C-14-ether (*2*) as the tracer to analyze the reaction product.

## METHOD

The esterification reaction between acrylic acid and ethyl alcohol may be driven to completion in a number of ways. The technique used in this study was to carry out the reaction in the presence of a large amount of benzene (*22*). The benzene acts as a water-entraining fluid and both water and benzene are removed from the reaction zone by distillation. The water is separated in a phase separator and the benzene returned to the reaction mixture. When no more water is observed in the benzene distillate, the reaction is considered complete.

The amount of ether in the reaction mixture is then determined by applying the isotope dilution technique (*3*). Briefly, this technique consists of adding to the reaction mixture a measured amount of the ether to be determined containing a known amount of radioactivity. The ether is then isolated in a pure form and the activity measured. The dilution of activity by the nonradioactive ether in the mixture is a measure of the amount of that compound present. The important factor in this technique is that only enough material need be isolated to prepare samples for radioactivity measurements. The steps in the procedure were:

1. A measured amount of radioactive ether of known activity was added to the reaction mixture.
2. Diethyl ether was carefully distilled from the mixture by means of an efficient fractionating column.
3. The activity of the ether thus isolated was determined.
4. The data obtained were substituted into the standard isotope dilution equation

$$b = a(Z - 1)$$

where $b$ represents the amount of inactive material present, $a$ the amount of active material added, and $Z$ the ratio of the specific activity of the active ether to that of the ether sample isolated.

Five esterification reactions were carried out (Table I). The first two runs were 1-mole syntheses and in each the reaction mixture was predistilled to allow isolation of approximately 75 ml. of the lowest boiling portion of the mixture. The radioactive ether was added to this distillate and the distillation procedure described in step 2 was applied. Thus, the first two experiments

#### Table I. By-product Ether Formed during Direct Esterification of Acrylic Acid

| Expt. | Batch Size, Moles | Catalyst | Activity[a] of Radioactive Ether Added, C./M. | Active Ether Added, G. | Activity[a] of Ether Isolated from Reaction, C./M. | Ratio Activity Ether Added/Ether Isolated | Diff. in Counting Rate, % | Ether Present in Reaction Mixture, G. | Ether Based on Product Expected, % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | H$_2$SO$_4$ | 5790 | 0.8331 | 5360 | 1.084 | 7.5 | 0.0683 | 0.07 |
| 2 | 1 | H$_2$SO$_4$ | 5530 | 1.3396 | 5120 | 1.079 | 7.3 | 0.1058 | 0.11 |
| 3 | 2 | H$_2$SO$_4$ | 6780 | 1.4191 | 5770 | 1.174 | 14.8 | 0.2469 | 0.12 |
| 4 | 2 | p-Toluenesulfonic acid | 6780 | 1.4178 | 6230 | 1.089 | 8.2 | 0.1262 | 0.06 |
| 5 | 2 | Sulfonic acid ion exchange resin Amberlite IR 105 | 6780 | 1.4215 | 6510 | 1.042 | 4.0 | 0.0597 | 0.03 |

[a] All radioactivity measurements were made in a windowless, gas-flow Geiger-Müller counter. Counting rates were determined for infinitely thick layers of barium carbonate. These values, proportional to the specific activity of the samples, were used directly in all calculations.

#### Table II. Known Values in Evaluation of Experimental Techniques

| Benzene, G. | Inactive Ether Added, G. | Radioactive Ether Added, G. | Activity of Radioactive Ether, C./M. | Activity of Isolated Ether Calcd., C./M. | Found, C./M. | Diff., % | Ether Present (Based on Total Benzene), % Calcd. | Found |
|---|---|---|---|---|---|---|---|---|
| 450 | 0.6995 | 1.3944 | 6780 | 4510 | 4420 | +2.00 | 0.16 | 0.16 |
| 450 | 0.3247 | 1.3981 | 6780 | 5500 | 5400 | +1.82 | 0.07 | 0.08 |
| 450 | 0.1333 | 1.4164 | 6780 | 6210 | 6280 | -1.13 | 0.03 | 0.02 |

included a preliminary distillation involving a condensate which was predominantly benzene and which froze on the total condenser when solid carbon dioxide was used as the refrigerant. This necessitated the use of ice in the condensing unit and it was felt that under these conditions a portion of the ether might have escaped through the condenser. For experiments 3, 4, and 5 the procedure was changed by doubling the size of each batch and adding the radioactive ether directly to the reaction mixture.

#### RESULTS AND DISCUSSION

The results of the isotope dilution analyses (Table I) show that the amount of ether formed in these preparations is very small. To reduce the experimental data to a common basis, the amount of ether present was calculated as percentage based on the theoretical yield of ester (100 grams for a 1-mole batch). On this basis, all results are roughly of the same order of magnitude.

Because of the high volatility of diethyl ether and the numerous uncontrolled factors involved in these experiments, it does not necessarily follow that the differences in amounts of ether noted in experiments 3, 4, and 5 mirror solely a difference in activity of catalyst used. That this is undoubtedly a factor, however, is evidenced by the close agreement between all the experiments using sulfuric acid.

The sensitivity of the method used is a function of the batch size. This is shown by comparing the results of experiment 3, a 2-mole batch, with those of experiment 2, a 1-mole run. A device for increasing the sensitivity of the method, therefore, lies in increasing the size of the batch as far as is practical, or increasing the specific activity of the tracer ether.

The isotope dilution procedure is a very effective analytical method for detection of ethers in the presence of esters. The results are well outside statistical variation, but it was felt that a series of "knowns" should be run to determine whether the differences noted could be due to experimental manipulation. Accordingly, a volume of benzene approximately equivalent to the finished volume of a 2-mole run was placed in a distilling flask and a known amount of nonactive ether added. Radioactive ether in known quantity was then introduced, the mixture was carefully distilled, and a pure sample of ether was isolated. The activity difference between the radioactive ether added and the ether isolated was noted in the usual way and the amount of nonactive ether present was calculated by the standard isotope dilution equation. The data (Table II) indicate that observed differences in counting rates are very close to the calculated variations. The experimental differences presented in Table I are greater than the "technique" differences shown in Table II, which tends to confirm the presence of small but definite amounts of ether in the esterification reaction mixtures.

Careful distillation studies on pure ether, benzene-ether mixtures, and reaction product–ether mixtures showed no azeotrope formation. Ether distillation from the reaction, therefore, is obviously similar to the distillation of ether from benzene. Active ether distilled from benzene as in the knowns above was collected and oxidized, and the results were compared with undistilled active ether as follows:

Undistilled ether                 6680 c./m.
Ether distilled from benzene      6680 c./m.

As the same distilling column was used for all distillations, cuts of both active and diluted materials were directly comparable. These data establish the purity of the ether collected.

#### EXPERIMENTAL

**Preparation of Esters for Isotope Dilution.** For the preparation of a 2-mole batch, a 1-liter round-bottomed flask was charged with 144 grams (2 moles) of 99% acrylic acid, 50 grams of 95% ethyl alcohol, 1.0 gram of pyrogallol, and 0.4 gram of cuprous bromide as polymerization inhibitors, 200 ml. of benzene, and an appropriate amount of catalyst. The latter amounted to 1 gram when sulfuric acid was used, 4.0 grams when p-toluenesulfonic acid monohydrate was employed, and 33 grams of resin containing 54.5% moisture when Amberlite IR-105 was the catalyst. The flask was then fastened to a phase separator and cyclicly distilled with intermittent removal of a water-rich layer of distillate. The density of this layer was taken and its water content was calculated by means of the equation:

Grams of water =
$$\frac{(\text{density of layer} - 0.79) \times \text{grams of water-rich layer}}{0.21}$$

As water evolution decreased, ethyl alcohol was added in increments of 20 grams until a total of 135 grams had been introduced into the reaction. Distillation was continued until 90 to 100% of the theoretical amount of water had been taken out of the system. The mixture was then cooled in preparation for isotope dilution analysis.

**Isotope Dilution and Isolation of Ether.** To the reaction mixture, or to the distillate from the reaction, an appropriate, weighed amount of radioactive ether was added in a sealed ampoule. This ampoule was broken under the surface of the liquid in the flask and the resulting system was carefully distilled using a concentric tube fractionating column (7) provided with a distilling head having a cold finger charged with solid carbon dioxide. The column was allowed to operate at total reflux until it reached equilibrium (approximately 2 hours) and the distillate coming over at 33° to 34° C. was collected for activity evaluation.

For the investigation of the knowns, the same procedure was used, except that the distillation flask contained 450 grams of pure benzene in lieu of reaction mixture. To this was added a known amount of nonactive ether (also by means of sealed ampoules) before the introduction of the radioactive tracer.

**Radioactivity Measurements of Ether Samples.** In order to measure the radioactivity of the ether samples, the compounds

were oxidized using the Van Slyke–Folch reagent (9, 19) and the carbon dioxide was collected in carbon dioxide–free sodium hydroxide and precipitated as barium carbonate. The counting rates were determined for "infinitely" thick uniform layers of barium carbonate. Since these values are proportional to the specific activity of the burned fractions, they were used directly in all activity comparisons. All counting measurements were made in a windowless, gas flow Geiger-Müller counter connected to a conventional scaler. The counting time for each sample was made long enough to give a "reliable" error (9/10) of less than 1%.

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# Measurement of Atmospheric Pollution by Ultraviolet Photometry

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**A sensitive portable ultraviolet photometric analyzer is an extremely convenient device for determining the concentration of toxic gases and vapors in the atmosphere, but instruments described in the literature have generally been limited to one analytical wave length, 254 mμ, and data on the absorptivity of gases and vapors, on which analyzer calibrations could be based, have been scanty. A portable ultraviolet gas and vapor analyzer, based on Hanson's design, has been gradually improved during several years' experience with a number of these instruments at several chemical manufacturing plants. The wave-length range of the analyzer has been extended by relatively simple techniques, thereby enhancing sensitivity and minimizing interference. A convenient gas-mixing apparatus for calibrating sensitive gas analyzers under simulated operating conditions at concentrations from 20% to 0.1 p.p.m. has been developed. The extended wave-length range and the use of the calibrating apparatus have materially increased the utility and precision of the portable ultraviolet analyzer in the analysis of toxic gases and vapors in the atmosphere.**

IN THE measurement of atmospheric pollution by toxic gases and vapors, ultraviolet photometry has a number of important advantages over many alternative methods (2). It is extremely sensitive: Considerably less than the maximum allowable concentration of many atmospheric contaminants can be detected. Moreover, the equipment is relatively simple, and its rapid response permits measurements to be made in less than 1 minute. Over the past 15 years, a number of sensitive ultraviolet photometers for atmospheric pollution analysis have been described, notably by Hanson (3), Klotz and Dole (4), and Silverman (8). The great utility of such instruments has apparently not been widely appreciated, however, as commercial equipment of this type has been unavailable until very recently.

Portable ultraviolet analyzers previously described have provided only one analytical wave length, usually 254 mμ; convenient methods of calibrating them at low concentrations have been lacking; and published data on gas and vapor absorptivity, on which a calibration might be based, have been scanty. These limitations have made these analyzers difficult to apply to specific problems.

## DESCRIPTION OF INSTRUMENT

The type of analyzer described is similar in principle to one previously described by Hanson (3) but has been modified extensively to make it useful for the detection of a greater number of substances and to permit easier operation and maintenance. The principal parts of the instrument are an ultraviolet source having 80% of its measurable radiation at 254 mμ, a double-beam optical system, Type 935 vacuum-phototube detectors, and a sensitive, stable electronic microammeter. A similar instrument was for a time manufactured by the Mine Safety Appliances Co., but was discontinued several years ago. The instrument is currently manufactured by Manufacturers Engineering & Equipment Corp., Hatboro, Pa.

The analyzer is shown in Figure 1. The sample inlet and outlet are conveniently accessible on the front. On the top are the meter which indicates the absorbance of the sample, the range selector switch which permits the selection of full scale absorbances between 0.0005 and 0.05, the zero adjust for setting the zero of the microammeter, the scale adjust for setting the microammeter sensitivity, and coarse and fine optical balance controls for zeroing the instrument on a sample of nonabsorbing gas.

Figure 2 is a schematic diagram of the optical system and the photometric circuit. The right-hand or measuring phototube views the lamp through the sample cell, while the left-hand or reference phototube views the lamp directly, or through a wire screen used to achieve approximate balance of the phototube responses. Two screw-operated metal rods, a thick one in front of the reference phototube, and a thin one in front of the measuring phototube can be advanced across the faces of the phototubes, to serve as coarse and fine optical balance controls, respectively.

The simple photometric circuit makes use of a sensitive electronic microammeter to measure the difference in current between the two phototubes. The electronic microammeter has five full-scale ranges between 0 to 0.01 and 0 to 1.0 $\mu$a. The analyzer calibration in terms of absorbance depends directly on the magnitude of the photocurrent at zero concentration reading, so that the lamp intensity must be controlled in order to maintain constant sensitivity. The constant-current lamp circuit operating from a constant voltage supply, as shown in Figure 2, maintains constant lamp intensity. The phototubes are operated at a current of 10 $\mu$a. to permit the instrument to measure absorbances between 0 to 0.00043 and 0 to 0.046 (0.1% and 10% light absorption) full scale. The minimum detectable absorbance is 0.00004, corresponding to 0.01% absorption, a limit imposed by residual instability in the optical system.



**Figure 1. Portable ultraviolet gas analyzer**

An electric heater around the outside of the lamp prevents random condensation of mercury droplets inside the envelope. If this condensation is not prevented, it adversely affects analyzer stability especially since the two phototubes view opposite sides of the lamp. To permit a check of the full scale absorbance of the analyzer, there is included between the lamp and the sample cell a movable frame operated by an external control, by means of which a thin wire producing a known absorbance of 0.003 may be inserted into the beam.

### GASES AND VAPORS DETECTED

Table I lists the minimum concentrations of a number of gases and vapors detectable by the analyzer in a variety of possible operating conditions. The maximum allowable concentrations for 8-hour exposure are also listed (1). The gases and vapors listed are representative of the types of substances for which the instrument is useful.

In the normal operating condition using the total radiation of the germicidal lamp, the analyzer is extremely sensitive to mercury, ozone, benzene and its derivatives, ketones, chlorinated ethylenes, and sulfur dioxide, because of the strong absorption of these substances at 254 m$\mu$. However, because about 20% of the lamp radiation detectable with the Type 935 phototube is distributed mainly among the wave lengths 313, 365, 405, and 436 m$\mu$, the analyzer is also sensitive to nitrogen dioxide, carbon disulfide, chlorine, and bromine, which absorb strongly at these wave lengths. The sensitivity to this last group of compounds can be appreciably enhanced and the sensitivity to most of the former group reduced or eliminated by the simple expedient of inserting selected optical filters in the measuring beam, thereby limiting the analytical radiation to certain groups of wave lengths other than 254 m$\mu$.

The last three columns of Table I list the minimum detectable concentrations of a number of substances when filters are used. These data illustrate how the use of filters can conveniently increase the sensitivity to certain atmospheric contaminants while reducing the interfering effect of others.

The filters referred to in Table I are made from readily available glasses and liquid solutions. Filter A consists of two pieces

**Table I. Minimum Detectable Concentrations of Gases and Vapors Using Portable Ultraviolet Photometer**

| Substance | Maximum Allowable Concn., 8-Hour Exposure (1), P.P.M. by Vol. | Minimum Detectable Concn., P.P.M. | | | |
|---|---|---|---|---|---|
| | | No filter | Filter A | Filter B | Filter B with external meter |
| Acetone | 500 | 12 | 310 | 70 | 23 |
| Benzene | 35 | 0.8 | a | 9 | 3 |
| Bromine | 1 | 5 | 0.6 | 9 | 3 |
| Carbon disulfide | 20 | 45 | 54 | 24 | 7.5 |
| Chlorine | 1 | 25 | 7 | 7 | 2.3 |
| Chlorobenzene | 75 | 0.8 | a | 10 | 3 |
| Cyclohexanone | 100 | 25 | 210 | 70 | 23 |
| Mercury | 0.01 | 0.00003 | a | 0.0003 | 0.0001 |
| Nickel carbonyl | 1 | 0.015 | 0.7 | 0.15 | 0.045 |
| Nitrobenzene | 1 | 0.015 | a | 0.18 | 0.06 |
| Nitrogen dioxide | 25 | 2 | 0.3 | 1.7 | 0.55 |
| Ozone | 1 | 0.025 | a | 0.3 | 0.09 |
| Phosgene | 1 | 5.5 | a | 60 | 20 |
| Sulfur dioxide | 10 | 2.2 | 26 | 8 | 2.6 |
| Tetrachloroethylene | 100 | 1.0 | a | 12 | 4 |
| Toluene | 100 | 0.7 | a | 8 | 2.5 |

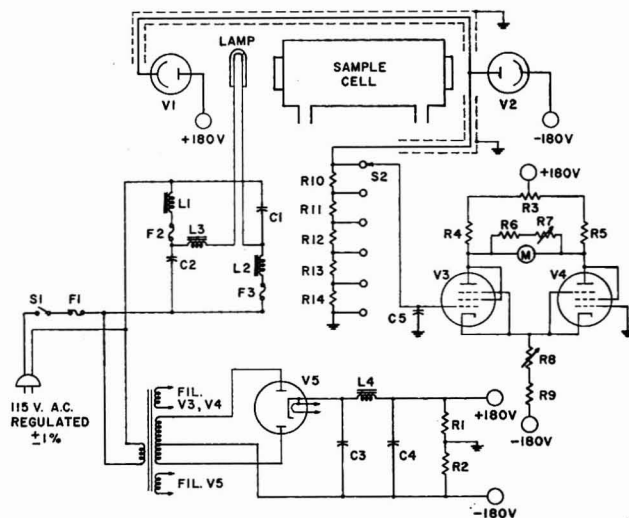$^a$ Not detectable at concentration levels found in atmosphere.



**Figure 2. Schematic diagram of analyzer**

C1, C2. Capacitor, paper, 1.5 mf., 2000 volts
C3, C4. Capacitor, electrolytic, 8 mf., 600 volts
C5. Capacitor, mica, 0.001 mf., 100 volts
F1. Fuse, 2 amperes
F2, F3. Fuse, instrument, 0.1 ampere
L1, L2. Choke, 5 henries, 100 ma., gap adjusted for series resonance with capacitor C1, C2, with lamp circuit open
L3. Choke, 5 henries, 100 ma.
Lamp. General Electric germicidal lamp, Type G4T4/1, 4 watts. Upper ¹/₃ of lamp are wound with 26 inches of Nichrome resistance wire, 0.020 inch in diameter, connected to 6.3-volt filament supply
M. Meter, 0-100 $\mu$a., 2000 ohms
R1, R2. Resistor, wire wound, 5000 ohms, 25 watts
R3. Potentiometer, 3000 ohms, zero control
R4, R5. Resistor, carbon, 25,000 ohms, 1 watt
R6. Resistor, carbon, 1000 ohms, ¹/₄ watt
R7. Rheostat, 5000 ohms, set-scale control
R8. Rheostat, 5000 ohms, bias control
R9. Resistor, carbon, 50,000 ohms, 2 watts
R10. Resistor, carbon, 10 megohms, ¹/₄ watt
R11. Resistor, carbon, 8 megohms, ¹/₄ watt
R12. Resistor, carbon, 1 megohm, ¹/₄ watt
R13. Resistor, carbon, 0.8 megohm, ¹/₄ watt
R14. Resistor, carbon, 0.2 megohm, ¹/₄ watt
S1. Switch, toggle, SPST, 5 amperes, 120 volts, on-off
S2. Switch, selector, 6-position, range control
V1, V2. Phototube, RCA Type 935
V3, V4. Tube, Type 6J7
V5. Tube, Type 5Z4

of Corning glass CS-9-53, available from Corning Glass Works, Corning, N. Y. Filter $B$ consists of a glass cell made by cementing or clamping filter-glass windows to a cylindrical cell body of borosilicate glass, 5 cm. in diameter and 1 cm. thick, having a glass-stoppered side arm for filling. One window is one half standard thickness of Corning Glass CS-9-53, the other is one half standard thickness of Corning Glass CS-7-54. The cell is filled with a 0.05 gram per liter solution of potassium chromate.

Table II gives the approximate spectral composition of the radiation as detected by the phototube both in the standard instrument without filters and when using each of the filter combinations given in Table I. The use of filters lowers the photocurrent and increases the full scale absorbance in inverse proportion, but the minimum detectable absorbance remains 0.00004 if the photocurrent is above 1 $\mu$a. For photocurrents less than 1 $\mu$a., the minimum detectable absorbance increases in inverse proportion to the current because of the limited sensitivity of the electronic microammeter. The external connection of a more sensitive microammeter, several of which have become commercially available since the analyzer was built, overcomes this limitation and permits a threefold decrease in the minimum detectable concentration of chlorine and carbon disufide (to 2.3 and 7.5 p.p.m., respectively).

### Table II. Spectral Energy Distribution in Portable Ultraviolet Analyzer under Various Operating Conditions

| Wave Length, M$\mu$ | Relative Measurable Energy, % | | | |
|---|---|---|---|---|
| | No filter | Filter A | Filter B | Filter B with external meter |
| 254 | 81 | a | 22 | 22 |
| 297 | 0.7 | 1.4 | 8 | 8 |
| 313 | 2.2 | 7.9 | 46 | 46 |
| 334 | 0.5 | 2.4 | 7 | 7 |
| 365 | 2.5 | 14 | 5 | 5 |
| 405 | 3.7 | 20 | 12 | 12 |
| 436 | 7.2 | 39 | a | a |
| 546 | 2.4 | 13 | a | a |
| 578 | 0.3 | 2 | a | a |
| Total photocurrent, microamperes | 10 | 1.5 | 0.32 | 0.32 |
| Full scale absorbance at most sensitive meter range | 0.00043 | 0.0029 | 0.013 | 0.0026 |
| Minimum detectable absorbance | 0.00004 | 0.00004 | 0.00013 | 0.00004 |

a Less than 0.01%.

Table III lists the approximate concentrations producing full scale deflection of the analyzer both for the normal analyzer, an analyzer using filters, and an analyzer using filters and an extra-sensitive external vacuum-tube microammeter. The external meter is assumed to read 0.002 $\mu$a. full scale. This is conveniently provided by a Keithley Model 200 electrometer with an attached shunt of $10^{-9}$ ohm (both available from Keithley Instruments, Cleveland, Ohio).

Table II shows that the analyzer is never operated with completely pure—i.e., monochromatic—radiation. Normally in spectrophotometry this practice is avoided, as it can sometimes cause large apparent deviations from the Bouguer-Beer law (5). In using this analyzer for measurement of atmospheric pollution, however, the absorbance of the atmospheric sample is usually so low that no appreciable deviations from the law occur at any concentration normally encountered. In the least favorable case, the detection of chlorine using unfiltered radiation, most of the radiation is at wave lengths only weakly absorbed, so that the calibration becomes nonlinear at an apparent absorbance of about 0.007. This corresponds to a concentration of 5000 p.p.m., several orders of magnitude higher than the maximum allowable concentration in the atmosphere. Similar effects occur when detecting carbon disulfide and nitrogen dioxide using unfiltered radiation but only above 10,000 and 1000 p.p.m., respectively.

### Table III. Concentrations of Gases and Vapors Giving Full Scale Meter Reading on Portable Ultraviolet Analyzer

| Substance | Concentration for Full Scale Deflection, P.P.M. by Vol. | | | |
|---|---|---|---|---|
| | No filter | Filter A | Filter B | Filter B with external meter a |
| Acetone | 123 | 31,000 | 7100 | 1420 |
| Benzene | 8.1 | .... | 920 | 184 |
| Bromine | 32 | 60 | 890 | 178 |
| Carbon disulfide | 470 | 5,400 | 2340 | 468 |
| Chlorine | 270 | 670 | 750 | 150 |
| Chlorobenzene | 8.4 | .... | 960 | 192 |
| Cyclohexanone | 230 | 21,000 | 7100 | 1420 |
| Mercury | 0.0003 | .... | 0.03 | 0.6 |
| Nickel carbonyl | 0.15 | 67 | 14 | 2.8 |
| Nitrobenzene | 0.16 | .... | 18 | 3.6 |
| Nitrogen dioxide | 14.3 | 29 | 170 | 34 |
| Ozone | 0.25 | .... | 27 | 5.4 |
| Phosgene | 55 | .... | 630 | 126 |
| Sulfur dioxide | 22 | 2,600 | 810 | 162 |
| Tetrachloroethylene | 10.3 | .... | 1180 | 236 |
| Toluene | 7.0 | .... | 800 | 160 |

a Assuming external meter reads 0.002 $\mu$a. full scale.

### OPERATION OF ANALYZER

In use, the analyzer is carried to the site where measurements are to be made and plugged into the alternating current line, through a constant-voltage transformer. The sample outlet is connected to a convenient source of vacuum such as a small pump or aspirator and the instrument is allowed to warm up. Readings can be made on the most sensitive scale in 30 minutes, although maximum stability is not reached until after a 1-hour warmup. Before each reading the instrument is standardized by introducing a nonabsorbing sample, such as air or nitrogen, and adjusting the optical controls until the instrument reads zero absorbance. A small bottle of compressed air or nitrogen is the most convenient source of standardizing gas. In the original instrument of Hanson, an activated charcoal filter was used to clean the ambient air for standardization. This procedure, while satisfactory in the determination of chlorinated ethylenes and certain other solvents, is not adequate for all the substances for which the instrument is currently used; the procedure of standardizing on bottled gas is more generally applicable.

To sample a suspected atmosphere, a sampling tube is run from the analyzer inlet to the contaminated area. The tubing material should be one which does not have a strong tendency to adsorb vapors. Glass tubing is good in this respect, but is inconveniently fragile. Saran is the most generally satisfactory material, as it is flexible enough for convenient probing but is not a strong adsorber of vapors. Rubber and most plastic tubing are generally unsatisfactory because of their great tendency to adsorb vapors; when used as sample tubing, they greatly lengthen the apparent response time of the analyzer because of the time required to saturate the tubing.

Care must be exercised to avoid exposing the instrument needlessly to very high concentrations of vapors, such as the atmosphere in the top of a solvent container, as a long air purge is sometimes required to remove the vapor adsorbed on the cell walls during such exposure.

For very dusty samples a filter of glass wool or glass cloth may be necessary. It should, however, be used with discretion, as its large surface area may adsorb a low concentration of vapor.

In cases where a number of detectable vapors are present simultaneously and differentiation is required, some success has been attained with differential measurement before and after a selective scrubbing of the sample. Selective scrubbing action at low concentrations is not always predictable but with the aid of the calibrating apparatus described, it is a fairly simple procedure to test a selective scrubbing system for complete removal of one component and nonremoval of another.

The long warmup time of the analyzer is inconvenient when many different sites must be investigated. A solution to this problem used in one plant is to mount the analyzer on a hand cart with storage batteries and vibrator for supplying 115 volts alternating current, as well as a standardizing valve, a bottle of standardizing gas, and a pump. If desired, a manifold and

absorption trains for selective scrubbing may also be carried. The use of the cart permits the analyzer to be energized continuously while it is moved around the plant, and it eliminates the inconvenience of the long warmup time.

It is desirable to check the sensitivity of the analyzer occasionally, because a change of sensitivity can arise from two causes: decrease in light source intensity and variation in its spectral purity. The former is detected by means of the fine wire calibrator and the readings can be compensated for any change by the adjustment of the "set scale" control. Purity variation is more troublesome, especially at the wave length of 254 mμ. The formation of obscuring deposits on the lamp envelope causes a disproportionate reduction in the intensity of this line, with a consequent change in spectral purity. The purity can conveniently be measured by the use of a Corning Glass CS-9-53 filter, which is opaque to 254 mμ radiation, but transmits radiation of longer wave length from a mercury arc with only an 8% loss due to surface reflection. When the current of one of the phototubes is measured, both before and after the insertion of this filter in the optical path, the purity of the source radiation, as seen by the phototube, is calculable as $100(I_0 - 1.1 I_F)/I_0$, where $I_0$ and $I_F$ are the phototube currents before and after the insertion of the filter, respectively. This measurement can be made conveniently in the analyzer with the front panel removed.

Connect a shunt of approximately 20,000 ohms across the input of the electronic microammeter to reduce its sensitivity to about 10 μa. full scale, place an opaque screen in the reference beam to permit direct measurement of the current of the measuring phototube, and read the current before and after the insertion of the filter in the measuring beam. The span of the instrument in units of concentration of the component sought may then be corrected, the exact procedure differing depending on whether the compound absorbs mainly at 254 mμ—e.g., benzene, ozone—or mainly at longer wave lengths—e.g., chlorine, nitrogen dioxide. In the former case, the full scale concentration is multiplied by the ratio (new purity)/(old purity); in the latter case, the multiplier is (100 − new purity)/(100 − old purity). Among new lamps differences are sometimes found in the relative proportions of radiation at 254 mμ and at longer wave lengths. The above method of purity measurement and compensation may also be applied in these cases.

## CALIBRATION PROBLEMS

As in all inferential analytical methods at low concentrations, calibration is one of the most difficult problems in the application of the portable photoelectric analyzer. Published data on the spectral absorptivity of vapors are still rather scanty. Where no other data are available, the molar absorptivity of a gas or vapor may be assumed to equal the molar absorptivity of the substance in liquid solution in a nonpolar solvent, such as hexane. This assumption gives results which are within a factor of 3 of the true absorptivity of many compounds. However, in a device like the portable analyzer, using a few wave lengths of radiation selected only because they are conveniently available from a mercury arc, this assumption may sometimes lead to serious error. A substance which in liquid solution shows a high absorptivity at one of these wave lengths may as a dilute vapor exhibit spectral fine structure with a much lower absorptivity at that same wave length. This effect, which is most serious, of course, with aromatic hydrocarbons and their derivatives, is amplified by the high spectral resolution provided by a line source of radiation like the mercury arc. Because of the difficulty in predicting the calibration of the analyzer for a specific substance, an empirical calibration over the desired concentration range is generally desirable.

Attempts have been made to prepare calibrating mixtures of vapors by vaporizing measured amounts of volatile liquids in large containers of air. At low concentrations, this method is subject to large errors resulting from loss of the vapor by adsorption on the walls of the container or its connections. Rough

metal and rubber or plastic tubing are the most serious source of this sort of trouble, but, even in an all-glass system, the author's experience indicates that 100 p.p.m. by volume is about the lowest concentration that can be prepared reliably by this method.

## CALIBRATING APPARATUS

It seemed evident that the only way to obtain calibrating mixtures having concentrations as low as 1 p.p.m. was by the use of an all-glass system in which a flowing mixture of gas or vapor in air is continuously prepared. The all-glass construction insures that vapor adsorption is small, while, if the continuous flow is made large enough, equilibrium between the glass walls and the sample is quickly attained and any errors due to vapor adsorption are minimized.
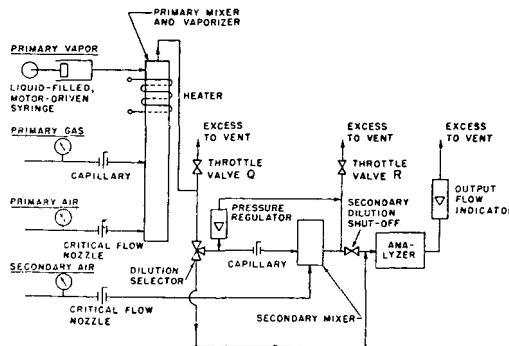


**Figure 3.  Schematic diagram of calibrating apparatus**

An apparatus has been constructed that can supply a continuously flowing calibrating mixture of constant preselected composition at a rate of 500 ml. per minute. A schematic diagram of the apparatus is shown in Figure 3. Two-stage dilution is used to permit a wide selection of concentrations. Relatively high concentrations of gas or vapor are first prepared by mixing with air in the primary mixer, then lower concentrations are prepared by further mixing with air in the secondary mixer.

Positive metering of gas and air is achieved by regulating the pressure drop caused by the flow through a constriction meter. For metering air, critical flow nozzles (7) are used because of the convenient linear relation between flow and pressure. For other gases, long viscous-flow capillaries are used because their calibrations are more readily converted from one gas to another (6). The all-glass construction permits easy inspection of the constrictions for partial plugging, and thus eliminates one of the chief objections to this type of flowmeter. The possibility of plugging is minimized by passing the gas or air through glass-frit filters ahead of the constriction meters. The regulated primary air pressure may be set between 10 and 60 pounds per square inch gage; the regulated gas pressure is set at 17 pounds per square inch gage. The pressure downstream from the constrictions is nearly atmospheric and small changes in this pressure do not affect the metered flow.

Positive metering of the liquid introduced into the apparatus for vaporization is achieved by driving the piston of a calibrated hypodermic syringe at a slow speed with a synchronous motor and gear train. The liquid is then vaporized in an electrically heated, wetted-wall evaporator.

By a choice of two gas capillaries or three syringes in conjuction with three primary-air nozzles, it is possible to prepare concentrations from about 100 p.p.m. to about 20% by volume (or in the case of vapors nearly to saturation).

The primary mixture may be used directly if medium or high concentrations are desired. If concentrations lower than 100 p.p.m. are needed, the primary mixture may be further diluted with air in the secondary mixer. The supply of primary mixture to

the secondary mixer is metered at low pressure drop (a few inches of water) by means of a capillary in combination with a small rotameter used as a sensitive differential pressure meter. Air for secondary dilution is provided through a metering system similar to that used for primary air. Two air nozzles in combination with two capillaries supplying primary mixture permit a choice of dilution from 10 to 1400 times. With double dilution, concentrations as low as 0.1 p.p.m. are obtainable.

With liquids of low vapor pressure (1 mm. of mercury or lower), it may not be possible to prepare successfully the minimum concentration of 100 p.p.m. normally obtainable in the primary mixer. In such cases, a dilute solution of the material in a solvent such as water or ethyl alcohol, which is transparent to ultraviolet radiation, may be successfully vaporized in the primary mixer. Then, if desired, the concentration can be reduced by secondary dilution.

The apparatus is mounted on a board approximately 1.2 × 1.1 meters (4 × 3.5 feet). A graphic flow diagram on the board together with instructional labels at each valve simplifies the operation of the apparatus by nontechnical personnel. All of the constriction meters are connected to the apparatus by spherical joints to permit easy removal for cleaning.

The precision of the apparatus was tested by making 44 measurements of the molar absorptivity of acetone vapor at concentrations from 400 p.p.m. to 17%. In a plot of absorptivity vs. concentration, all data fell on a smooth curve within a standard deviation of ±5%, a precision sufficient for most measurements of atmospheric pollution.

The apparatus can be set up for a calibration run on a gas or vapor in about 20 minutes. As it is easily cleaned, calibrations for eight different gases and vapors can be made in one working day. The apparatus has been in use over 4 years and has been a useful tool in testing the performance of the portable ultraviolet analyzer and other sensitive analytical instruments.

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# Spectrophotometric Determination of Bismuth with Ethylenediaminetetraacetic Acid

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In the search for further applications of ultraviolet spectrophotometry in inorganic analysis, the spectral characteristics of various metal complexes with ethylenediaminetetraacetic acid were studied. The absorption band of the bismuth complex, at 263 m$\mu$, was found suitable for the quantitative determination of this element. The procedure evolved permits the determination of bismuth in a concentration range from 2 to 25 p.p.m. in weakly acidic solution or at a pH of 1. Iron(III) and copper(II) must be absent from solution. An excess of antimony(III), tin(II), mercury(II), or lead, in decreasing order, causes moderate interferences. The method is rapid and reliable and compares favorably with other spectrophotometric procedures for the determination of bismuth. The reagent used is stable, as is the complex on which the absorption measurements are based.

IN RECENT years a number of analytical methods, based on the formation of a stable bismuth ethylenediamine tetraacetate complex (bismuth Versenate) have been reported in the literature. These publications refer to indirect titrations (2), direct titrations (1), titrations utilizing spectrophotometric end points (8), amperometric (6, 7) and potentiometric (5) titrations, and gravimetric separations (4). The fact that bismuth Versenate exhibits a pronounced absorption maximum at a wave length of 263.5 m$\mu$ suggested the further possibility of applying this complex in a spectrophotometric determination of the element.

With this possibility in mind, the spectral characteristics, the influence of pH, and interferences from other cations present in solution have been studied; and a procedure for the determination of bismuth in a range of concentration from 2 to 25 p.p.m. has been evolved.

## APPARATUS

Spectrophotometric measurements were carried out by means of a Beckman Model DU spectrophotometer, using 1.00-cm. quartz cells. A Beckman Model G pH meter was applied for the determination of hydrogen ion activities.

## SOLUTIONS AND REAGENTS

All solutions were prepared from reagent grade chemicals. A 0.0200M bismuth stock solution was prepared by dissolving 2.330 grams of bismuth oxide in hydrochloric acid and diluting to 1 liter. The concentration of this stock solution was checked by spectrophotometric comparison of the Versene complex with a second standard solution which was obtained from 99.8% bismuth metal dissolved in hot concentrated sulfuric acid and diluted with distilled water to yield the desired concentration. The stock solution was subsequently diluted to the desired concentration, maintaining the acidities high enough to prevent precipitation of bismuth oxychloride.

A 0.020M disodium salt of ethylenediaminetetraacetic acid (Versene) stock solution was obtained by dissolving 7.447 grams of disodium versenate dihydrate [Bersworth (now Versene Chemicals, Inc.) analytical reagent grade, dried over concentrated sulfuric acid] in 1 liter of distilled water.

Solutions used in the studies on interferences were prepared by dissolving weighed amounts of appropriate metal salts in distilled water or hydrochloric acid. In cases where simple weighing of

the salts did not lead to the desired accuracy, standardizations of the solutions were made using established methods.

Buffer solutions were prepared as follows: Acetate buffer (pH 5.6) was made up to contain 0.75 mole per liter of sodium acetate and 0.25 mole of acetic acid per liter. A sulfate "buffer" was prepared by mixing 1.5 liters of saturated potassium sulfate solution with 1 liter of 2N sulfuric acid. A perchlorate buffer was obtained by dissolving approximately 200 grams of sodium perchlorate in 1 liter of 1N perchloric acid.

### EXPERIMENTAL

In order to study the influence of hydrogen ion concentration on the bismuth Versenate complex, the absorption spectra of four solutions were recorded (Figure 1). These solutions contained 0.5N perchloric acid, 0.5N hydrochloric acid, acetate buffer, and ammonia–ammonium chloride buffer, respectively. Studies at pH values above 10.3 (ammonia–ammonium chloride buffer) could not be made because increased alkalinity led to precipitation of bismuth. Each one of these solutions contained $8 \times 10^{-5}$ mole per liter of bismuth, and $10^{-3}$ mole per liter of disodium Versenate. Further, ten solutions of the same bismuth and Versene concentrations, ranging from 1.8N perchloric acid to slight alkalinity, were prepared, and their absorbancies were measured at 263.5 m$\mu$. In each case the blank contained equivalent amounts of Versene and acid. The values thus obtained, and likewise the peak values of the first set, were plotted against pH (Figure 2, curve I). Where equal concentrations of perchloric acid and hydrochloric acid led to appreciably lower results in the case of the latter, the absorbance values of the hydrochloric acid solutions were omitted in the plot, because these deviations were considered as resulting from the formation of the bismuth chloro complex (3) rather than from hydrogen ion concentration (compare Figure 1, plots I and II).
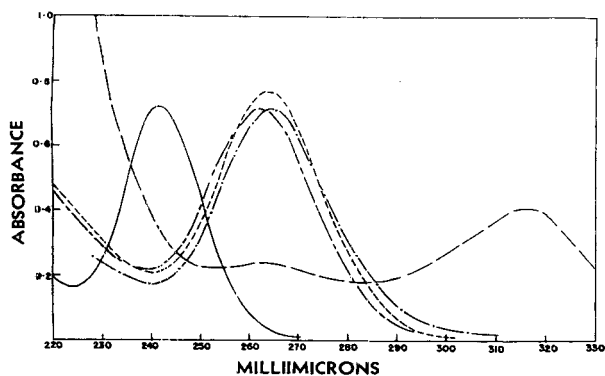


**Figure 1. Absorption spectra of bismuth and lead Versenate**

$8 \times 10^{-5}M$ bismuth chloride in $10^{-3}M$ disodium Versenate
I.    — — — — in 0.5N HCl
II.   — - — - — in 0.5N HClO₄
III.  - - - - - - at pH 4.5
IV.   — - — - — - at pH 10.3
$8 \times 10^{-5}M$ lead Versenate in $10^{-3}M$ disodium Versenate
V.    ———————— at pH 4.7

Absorption due to the complexing agent had to be considered. In analogy to the procedure described, the pH dependence of the absorbance of 0.002M disodium Versenate was determined at 241.5 m$\mu$ (Figure 2, curve II), and at 263.5 m$\mu$ (Figure 2, curve III).

It is evident from these plots that the peak values of adsorption as well as the background remain constant at 263.5 m$\mu$ from pH 2 to 9, the molecular extinction coefficient of bismuth Versenate being $9.35 \times 10^3$.

The required excess of complexing agent was determined by measuring the peak absorbance of nine weakly acidic solutions which contained fixed amounts of bismuth ($8 \times 10^{-5}$ mole per liter), but which varied in Versene concentration from $2 \times 10^{-5}$ to $4 \times 10^{-3}$ mole per liter; precipitated bismuth oxychloride was

removed by centrifugation. The shape of the plot (Figure 3) indicates great stability of the 1 to 1 bismuth Versenate *complex;* the limiting absorbancy is attained at equimolar mixtures of bismuth and Versene.

In determining the stability of the complex with respect to time, it was found that the readings were constant over a time interval from 5 minutes to 50 hours after the solutions were prepared. The temperature coefficient was calculated to be $-0.2\%$ per degree centigrade.

**Calibration.** Working curves were set up for acetate buffered solutions and for solutions buffered with sulfate (pH 0.9 to 1.1). At pH 1, the readings obtained for given concentrations of bismuth were found to be approximately 5% lower than the ones obtained from acetate buffered solutions, due to the incipient state of the dissociation of the bismuth Versenate. The medium of high acidity was chosen in order to minimize effects from interfering cations. The bismuth standards used in this calibra-

**Table I. Cation Interferences**

| | Approx. Amount (P.P.M.) of Interfering Ion Exhibiting Absorbance Equal to 0.1 P.P.M. Bismuth | | |
|---|---|---|---|
| Cation | Acetate-buffered solution | pH 1 | Note |
| Alkali metals | ... | .... | No interference |
| Alkaline earths a | ... | .... | No interference |
| Cadmium | ... | .... | No interference |
| Zinc | ... | .... | No interference |
| Aluminum | ... | .... | No interference |
| Arsenic(III) | ... | .... | May cause very slight negative deviations |
| Mercury(II) | 0.6 | 9 | Interference |
| Copper(II) | <0.1 | 0.1 | Heavy interference |
| Manganese(II) | 20 | >20 | Small positive deviations at high concentrations |
| Chromium(III) | 5 | >20 | Slight interference in acetate buffered solution |
| Nickel | 8 | .... | No interference at pH 1 |
| Cobalt | 3 | .... | No interference at pH 1 |
| Tin(II) | 1 | <5 | Interference |
| Tin(IV) | Precipitated | | |
| Antimony(III) | 1.1 | <3 | Interference |
| Iron(III) | <0.1 | <0.1 | Heavy interference |
| Lead | 2.5 | 15 b | Interference in acetate buffered solution |

a Presence of sulfate is to be avoided.
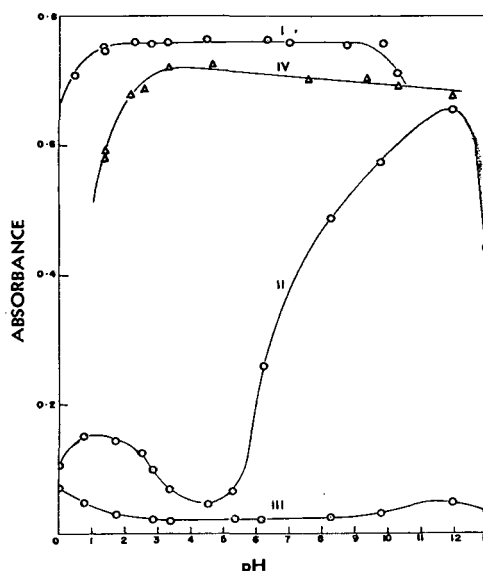b In perchlorate medium of pH 0.9.



**Figure 2. Change of absorbancy with pH**

I.    $8 \times 10^{-5}M$ bismuth chloride in $10^{-3}M$ disodium Versenate, at 263.5 m$\mu$
II.   $2 \times 10^{-3}M$ disodium Versenate at 241.5 m$\mu$
III.  $2 \times 10^{-3}M$ disodium Versenate at 263.5 m$\mu$
IV.   $8 \times 10^{-5}M$ lead chloride in $10^{-3}M$ disodium Versenate at 241.5 m$\mu$

tion were prepared by transferring 0.5 to 6 ml. of 0.001$M$ bismuth chloride solution into 50-ml. volumetric flasks. The Versene concentration was set at 0.001$M$. The blanks contained an equal amount of buffer and complexing agent.

It was found that the Beer-Lambert law was obeyed from 2 to 25 p.p.m. (within the limits of experimental error), the optimal concentration being 10 p.p.m., as established by a Ringbom plot of the calibration data.

**Reproducibility of Measurements.** The reproducibility of absorbancy measurements at 263 m$\mu$ of 17 solutions containing 6 $\times$ 10$^{-5}$ mole per liter of bismuth and sulfate buffer (pH 0.9 to 1.1) was checked over a period of 3 days. The absorbancies varied from 0.540 to 0.550, the average being 0.543 and the standard deviation $\pm$0.6%.

**Interferences from Cations.** In studies on interferences, varying amounts of potentially interfering ions were added to acetate buffered solutions and to sulfate buffered solutions in which the bismuth and Versene concentrations were kept constant. The deviations from the theoretical values for the respective concentration of bismuth were determined. Thus, approximate values for the extent of interferences were obtained and expressed as parts per million of interfering ion exhibiting the same absorbance as 0.1 p.p.m. of bismuth (Table I).

Because lead is precipitated as lead sulfate on addition of the sulfate buffer, it was necessary to remove such precipitates by centrifugation prior to determination of bismuth. Results obtained by this procedure are presented in Table II.

**Absorption Spectrum of Lead Versenate.** Finally the absorption spectrum (Figure 1, plot V), the pH dependence of the absorption maximum (Figure 2, curve IV), and a calibration curve in acetate buffered solution were determined for the lead Versene complex by analogous procedures. The molecular extinction coefficient at 241.5 m$\mu$ was found to be 8.8 $\times$ 10$^3$.

### DISCUSSION

Comparing plots I and III, as well as plots II and IV (Figure 2), it becomes evident that the conditions are favorable for a spectrophotometric determination of bismuth, while lead would require a careful control of pH because of the extensive variation of the Versene background with variation in acidity. Moreover, at a wave length of 241.5 m$\mu$, interferences from diverse cations and anions are so numerous that a spectrophotometric determination of lead as the Versenate does not appear to be generally practical.

Because it is known that Versene forms complexes with many metal ions, one would expect a number of interferences also in the determination of bismuth. The complexes of the alkaline earth metals, of zinc, cadmium, and aluminum show the same low absorption at 263 m$\mu$ as the reagent; yet their absorbancy at 241 m$\mu$ is lower than that of the disodium Versenate, thus causing negative deviations for measurements made at this wave length.

The interference from manganese(II), slightly positive, and from arsenic(III), slightly negative, can be considered as being negligible in a determination of bismuth. Chromium(III), nickel, and cobalt slightly interfere at pH 4 to 5, while mercury(II), tin(II), lead, and antimony(III) cause a marked increase in absorbancy. Iron(III) and copper(II), even if present in very low concentrations, cause serious interferences and must be absent.

If the acidity is increased to a pH of 1, an appreciable decrease in interference from bivalent ions is observed, due to the instability of their Versene complexes in acidic medium. The interference from copper(II) cannot be successfully obviated in this manner.

Comparing the figures in the first and second columns of Table I, it can be seen that determination of bismuth at pH 1 is to be preferred if other cations are present. The values listed represent only an approximate average calculated from deviations at different concentrations.

### Table II. Interference from Lead

(Lead precipitated in sulfate medium at pH 1; removed by centrifugation)

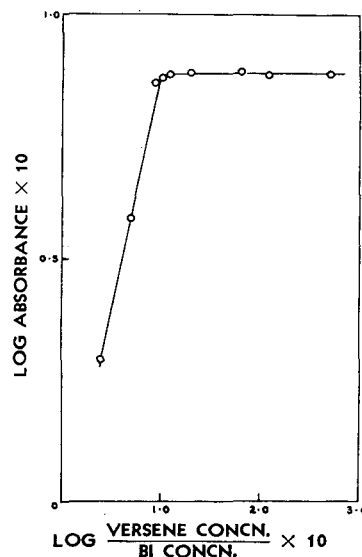| Bismuth Added, 10$^{-5}$ Mole/Liter | Lead Added, 10$^{-5}$ Mole/Liter | Bismuth Determined, 10$^{-5}$ Mole/Liter | Error, % |
|---|---|---|---|
| 4.0 | 50 | 4.14 | +2.5 |
| 6.0 | 50 | 4.03 | +0.8 |
| 8.0 | 50 | 8.12 | +1.5 |
| 4.0 | 200 | 4.02 | +0.5 |
| 6.0 | 200 | 5.90 | −1.7 |
| 8.0 | 200 | 8.00 | 0.0 |
| 4.0 | 500 | 3.90 | −2.5 |
| 6.0 | 500 | 5.95 | −0.8 |
| 8.0 | 500 | 7.60 | −5.0 |



**Figure 3. Values of maximum absorbancy (at 263.5 m$\mu$) of 8 $\times$ 10$^{-5}M$ bismuth at varying concentrations of disodium Versenate**

Lead and bismuth in acetate buffered solution, in the absence of interfering ions, may be determined simultaneously. The absorption minimum of the bismuth EDTA complex spectrum at 241.5 m$\mu$ closely approximates the Beer-Lambert law. However, an accurate determination of lead can only be achieved by rigorous control of experimental conditions. The concentration of bismuth, however, can be determined with higher accuracy than the concentration of lead, due to the fact that the extinction of lead Versenate at 263.5 m$\mu$ amounts to only one twenty-fifth of the extinction at 241.5 m$\mu$.

Lead is precipitated on addition of sulfate buffer, and the precipitate may be removed by centrifugation; under these conditions bismuth can be determined with reasonable accuracy, even if a considerable excess of lead had been present. Results obtained by such a procedure are presented in Table II. A small random error appears to be difficult to avoid. Indications were given that lead sulfate tends to adsorb bismuth Versenate, especially if the precipitate comes down in the form of very fine crystals. In order to avoid precipitation and associated difficulties, perchlorate buffer may be added (one fifth of the total volume) instead of sulfate buffer, in which case the approximate ratio of bismuth to lead extinction coefficient at 263.5 m$\mu$ was found to be 150 to 1.

As mentioned above, iron(III) and copper(II) must be absent from solution. An attempt was made to eliminate interference from iron(III) by reduction to iron(II) prior to determination, using hydrazine as the reducing agent. However, the theoretical value of absorbancy for pure bismuth Versenate could not be

attained. A more successful approach appeared to be elimination of iron(III) by repeated extraction of ferric chloride from 5N hydrochloric acid into ether. For this method it is essential that the volume of the sample be small and the bismuth concentration be above 0.001 mole per liter, thus permitting further dilution after extraction. Without final dilution after the extraction process, the salt concentration arising from the neutralization of hydrochloric acid becomes too high to allow an accurate determination of bismuth. If these requirements are met, one may remove a tenfold excess of iron(III) without an appreciable error.

With the exception of nitrate, common anions do not interfere. Nitrate, as is the general case in ultraviolet spectrophotometry, must be absent. Sulfate, perchlorate, acetate, and chloride have no effect, provided the latter is not present as a large excess of hydrochloric acid which may give rise to the formation of the bismuth chloride complex. (Compare plots I and II in Figure 1.)

## PROCEDURE

An appropriate aliquot of the unknown is transferred to a 50-ml. flask. The final concentration of bismuth should not exceed 25 p.p.m. Two and a half milliliters of 0.02M disodium Versenate are added. Because bismuth solutions generally contain large amounts of acid, it is necessary to neutralize excess acid by addition of ammonia, adjusting the final pH by means of sodium acetate. The solution is then diluted to the mark and its absorbance is measured against a blank containing an equal concentration of Versene. The blank should contain approximately the same amount of acid, ammonia, and sodium acetate as the unknown if the concentration of these reagents is high. The absorbancies should be measured at 263.5 mμ and the bismuth concentration be determined from the calibration curve. If it is desirable to reduce the effect of interfering cations by adjusting the acidity of the solution to pH 1, 25 ml. of sulfate buffer should be added instead of sodium acetate. The final pH of the sample should lie in the range of pH 0.8 to 1.2.

## CONCLUSION

A spectrophotometric procedure for the determination of bismuth by means of Versene is proposed. As to its practical significance as a method of ultraviolet spectrophotometry of bismuth, this method may compare with the determination of the element as the chloro complex (6); the latter method, however, has the advantage of measuring the absorbance at a considerably higher wave length which reduces the possibilities of interferences.

To apply an analogous procedure for the determination of lead does not appear to be justified from a practical standpoint except under special circumstances.

If bismuth is determined at a pH of 1, the presence of equal amounts of antimony(III) or tin(II) causes only slight interference, although heavy interferences from iron(III) and copper(II) still exist. In the presence of moderate excesses of lead, or in the presence of barium and strontium, a perchlorate medium is to be prepared over sulfate for controlling the pH. An excess of lead up to 50 times the concentration of bismuth may be eliminated by precipitation as the sulfate; higher concentrations coprecipitate bismuth.

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# Flame Spectrophotometric Determination of Copper in Nonferrous Alloys

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The Beckman Model DU flame spectrophotometer has been applied to the determination of copper in aluminum-, tin-, and zinc-base alloys. The copper arc emission lines in an oxyacetylene flame at 324.7 and 327.4 mμ were employed. The copper 324.7 mμ line is more sensitive and is recommended for copper concentrations less than 100 p.p.m. However, this line suffers more severely from self-absorption than does the copper 327.4 line, which is useful for concentrations of copper exceeding 100 p.p.m. Of all the elements commonly encountered in the above alloys, only appreciable amounts of nickel offered interference. The sensitivity of the flame spectrophotometric method is approximately 1 p.p.m. per instrument scale division. The standard deviations from the mean of replicate Bureau of Standards samples and from the certificate values are both within 3%. The flame analysis method compares favorably in precision and accuracy with conventional colorimetric methods in the concentration range 0.0 to 5.0% copper present. The time of analysis is shortened drastically to a few minutes following dissolution of the sample, as no preliminary separations are required prior to the flame analysis.

THIS investigation continues a series of reports from this laboratory on the application of flame spectrophotometry to the rapid determination of some of the components commonly present in alloys and ores. Although many papers have described methods for the determination of the alkalies and alkaline earths, relatively few discuss flame methods for any of the other elements. Spectroscopic methods employing an arc or spark, or combination thereof, have been described for most of the elements, but there are occasions when a less expensive array of equipment, such as a flame spectrophotometer, would suffice for analyses. Furthermore, the flame offers certain desirable attributes lacked by an arc or a spark.

Compared with an arc struck between carbon electrodes, the temperature of a flame is not particularly high. As a result, only a few of the metals which may be present in the sample will be caused to emit their characteristic radiation. This simplicity of flame emissions is one of the definite advantages of flame spectrophotometry, particularly when high concentrations of line-rich elements are present. Furthermore, in the flame where excitation occurs mainly as a result of collisions between atoms and molecules, some systems may be relatively strong compared to their emission in an arc or a spark. This is certainly true for easily excited elements. It is less difficult in the flame than in
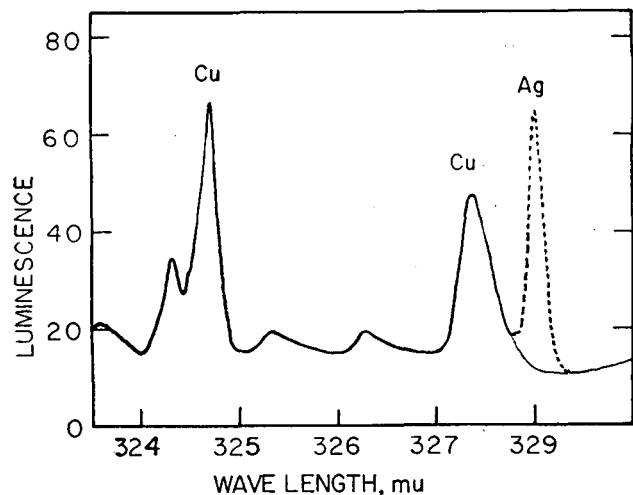
**Figure 1. General emission pattern of oxyacetylene flame containing copper and silver**

Present.   40 p.p.m. of copper
100 p.p.m. of silver

most other sources to establish reproducible conditions and so to obtain accurate quantitative determinations.

The element studied in this investigation is copper. It exhibits two rather sensitive emission lines in the ultraviolet portion of the spectrum, at 324.7 and 327.4 m$\mu$, and possesses a series of weak emission bands in the visible region (8). No thorough study has been reported which deals with the flame spectrophotometric determination of copper, although several investigators have employed the two lines mentioned to determine copper in biological materials (5) and in other substances (4). Lundegårdh (7) observed that the emission intensity or luminosity of copper was strongly dependent upon operating conditions, and that both lines were subject to strong self-absorption. The latter is not surprising in view of the low excitation potentials of these lines, the values being reported as 3.77 volts for copper 324.7 and as 3.80 volts for copper 327.4 (1). These conditions would seem to indicate that the flame spectrophotometric determination of copper might be something less than desirable. However, in the present study a general procedure is described which circumvents many of the previous difficulties.

For some types of samples, the simple measurement of the luminosity of a copper line minus the background luminosity suffices. On the other hand, many sample components enhance or suppress the line emissions of copper and the flame background to a different degree. When this situation exists, the well known spectroscopic principle of internal standard calibration offers a solution (2). The silver line at 328.0 m$\mu$ provides a satisfactory internal standard reference line. Although the spectrophotometer employed in this work was a single-beam type instrument, the flame conditions with an ordinary integral, metal aspirator-burner are sufficiently reproducible and constant for short periods of time to enable an operator to scan either or both of the copper lines, the silver line, and the background radiation immediately adjacent to the base of each of the respective lines.

Results obtained upon applying the flame spectrophotometric method for copper in various nonferrous alloys indicate that the method possesses a precision and accuracy within approximately 3%. Thus, the flame analyses compare favorably with conventional colorimetric methods in the lower concentration ranges, while drastically shortening the time of analysis to a few minutes following dissolution of the sample. For larger amounts of copper the time saved is even more significant, as conventional procedures usually require the preliminary separation of copper,

often as the sulfide, which is very time-consuming when compared to the rapidity of flame analysis.

## GENERAL EXPERIMENTAL WORK

**Apparatus.** A Beckman Model DU quartz spectrophotometer with Model 9220 flame attachment and photomultiplier unit was employed. A metal burner, constructed for use with oxygen and acetylene gases, was used in all experimental work.

To study emission intensities in various regions of the flame mantle, the burner was removed from its usual mounting and rigidly attached to the rack-and-pinion movement from a Duboscq colorimeter, and the whole assembly was mounted on a sturdy tripod. Provision was made for simultaneously raising or lowering a container which held the solution under investigation.

**Reagents.** A standard solution of copper, 1.00 ml. equivalent to 1.00 mg., was prepared by dissolving 3.942 grams of fresh crystals of reagent grade cupric sulfate pentahydrate in demineralized water, and diluting to 1 liter.

A standard solution of silver, 1.00 ml. equivalent to 2.00 mg., was prepared by dissolving 3.148 grams of silver nitrate, meeting ACS specifications, in demineralized water, and diluting to 1 liter.

Demineralized water, used exclusively in preparing all solutions and samples, was prepared by passing ordinary distilled water through a bed of Amberlite MB-3 resin.

**Flame Spectrophotometer Settings.** The instrument settings used for measuring the flame emissions were as follows:

| | |
|---|---|
| Sensitivity control | 5 or 6 turns from clockwise limit |
| Selector switch | 0.1 |
| Phototube resistor | 22 megohms |
| Slit | 0.030 mm. |
| Acetylene | 5 pounds per square inch |
| Oxygen | 6 pounds per square inch |
| Phototube voltage | 60 volts per dynode |

**Characteristics of Copper in Flame.** The general emission pattern of an oxyacetylene flame containing copper over the wave-length region 324 to 330 m$\mu$ is shown as Figure 1. Copper exhibits two arc lines at 324.7 and 327.4 m$\mu$. Also noticeable in Figure 1 are some band structures due to the flame itself. Weak remnants of the OH band system are observed around 324.3 m$\mu$, and some weak CO band heads appear at 325.3 and 326.3 m$\mu$. The remainder of the background radiation in the vicinity of the copper lines is essentially continuous and is attributable to the continuous spectrum of the carbon monoxide flame. This continuous background proves useful in analyses, as it can be employed as a form of internal standard or measure of flame constancy.

Both of the copper lines are components of the same doublet. They are low energy lines involving an electron which has been elevated from the lowest energy level, or ground state, to the next higher energy level. When the excited copper atom returns to its ground state, the two lines appear in emission. They are represented by these spectroscopic term symbols: $4^2S_{1/2}$ — $4^2P_{3/2}$ for the 324.7 line and $4^2S_{1/2}$ — $4^2P_{1/2}$ for the 327.4 line. Transitions from ground state to the most easily excited upper energy level, the 4P-level for copper, are known as resonance lines.

Resonance lines are prone to suffer self-absorption and copper lines are no exception. Self-absorption occurs during the passage of emitted radiation through the outer fringes of the flame mantle. Unexcited atoms of copper present in the outer portion of the flame mantle tend to absorb some of the copper radiation through interaction with the emitted light quanta. The absorbed copper light cannot therefore contribute to the observed luminosity. At relatively low concentrations self-absorption is not serious because the vapor density of unexcited copper atoms will be low. However, as the concentration of copper atoms injected into the flame increases, an increasing portion of the copper emission is absorbed before it reaches the periphery of the flame, and hence is not registered by the photosensitive detector of the spectrophotometer. The over-all effect on the luminosity of the two copper lines is shown in Figure 2, in which the luminescence of the two emission lines of copper is

plotted as a function of the concentration of copper injected into the flame.

The copper 324.7 m$\mu$ line is the more sensitive of the two lines for low concentrations of copper. Above approximately 75 to 90 p.p.m. of copper, the luminosity curve for this line flattens out rapidly until the slope of the copper 324.7 m$\mu$ line becomes less than the slope of the copper 327.4 m$\mu$ line. The onset of serious self-absorption does not occur with the latter line until the copper concentration exceeds 300 p.p.m. Consequently, the copper 327.4 m$\mu$ line is actually more sensitive and better suited for use when the concentration of copper present lies in the interval from 90 to 300 p.p.m.
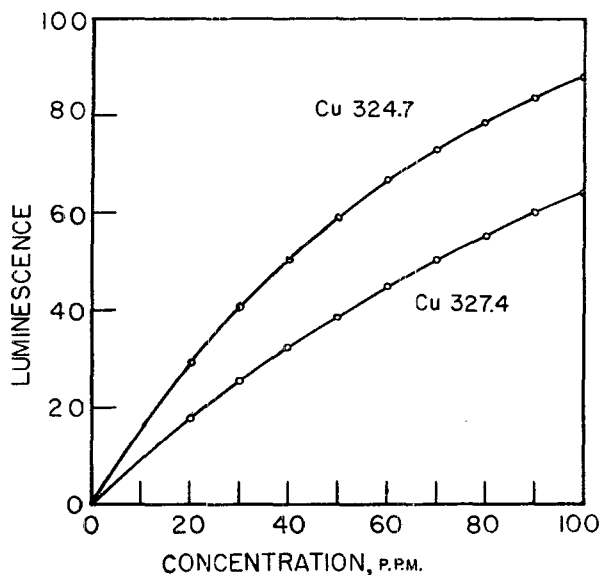


**Figure 2. Emission intensity of copper lines**

Luminescence given as instrument scale divisions

SLIT WIDTH. On the basis of Figure 1 it seemed likely that a slit width of 0.030 mm. was satisfactory. At this slit opening the effective band width of the emission lines was sufficiently narrow to enable a background reading to be obtained within 0.3 m$\mu$ of the wave length corresponding to the peak of the emission line. If the slit width were any wider, difficulty would be experienced in the region of the copper 324.7 m$\mu$ line due to the weak flame band centered around 324.3 m$\mu$, and in the vicinity of the copper 327.4 m$\mu$ line and the silver 328.0 m$\mu$ line due to their overlap. A photomultiplier attachment or equivalent amplification of the photocurrent is necessary in order to achieve reasonable response to luminescence at slit openings as small as 0.030 mm.

OPTIMUM FUEL PRESSURES. The relative intensities of flame emission lines are influenced by the ratio of oxygen and fuel pressures. Not always realized is that with an integral atomizer-burner, the oxygen pressure also influences the spray rate, and therefore, the flame temperature indirectly through variation in the amount of liquid aspirated into the flame per second. A study of the copper luminescence as a function of the two parameters, pressure of oxygen and of acetylene, revealed that the optimum operating conditions were pressures of 6 pounds per square inch of oxygen and 5 pounds per square inch of acetylene when a relatively wide orifice burner was employed (rated at a pressure of 10 pounds per square inch oxygen by the manufacturer). Relevant data are shown as Figure 3.

The portion of the flame mantle focused upon the entrance slit of the monochromator should also be considered (6). Figure 4 shows the luminescence as a function of distance from the tip for different oxygen-acetylene ratios. From these luminosity
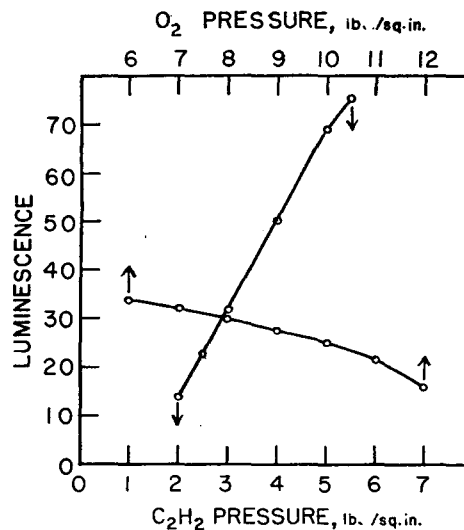


**Figure 3. Optimum fuel and oxygen pressures**

curves it is seen that the sensitivity of the copper lines depends to a large extent upon the position of the flame mantle in relation to the optical axis of the monochromator. None of the present commercial instruments provide means for altering the position of the flame, after initial optical alignment during assembly of the equipment, except in so far as different fuel flow or gas pressure may vary the position of the tip of the inner cone of the flame. The need for proper alignment is strikingly shown in Figure 4, which indicates that a considerable increase in copper luminosities reaching the photosensitive receiver would be accomplished through choice of optimum position of the flame mantle. Fortunately, the optimum position of the flame mantle, with respect to the entrance mirror on the monochromator, almost coincides with the normal position of the flame mantle when the burner is mounted in the usual manner in the burner housing of the Beckman instrument.

The traces in Figure 4 confirmed the original choice of fuel pressures, which were then maintained invariant throughout the remainder of the work. These fuel pressures gave a very hot flame, and although the data would seem to indicate that even a hotter flame is desirable in terms of emission sensitivities, burner maintenance then becomes a serious problem. The brass burner becomes sufficiently hot itself to volatilize the collodion coating covering the screw heads used to position the aspirator tube. Oxygen is then able to escape into the acetylene orifice and causes erratic flame conditions. As a general guide to selecting optimum fuel pressures with different burners, one should use those pressures which provide as hot a flame as possible, yet with a relatively low aspiration rate.

## PREPARATION OF CALIBRATION CURVES

Two types of standard curves were prepared. One consisted simply of the plot of the concentration of copper present against the luminosity observed at the peak of the respective copper line, $L$, from which value was subtracted the background reading, $H$. Typical of this type of curve is Figure 2. The choice of wave length from which to obtain the background reading, $H$, depends upon which other elements might be present. If none are present that emit lines at 325.0 and 327.0 m$\mu$, then these two wave lengths are suitable for use with copper 324.7 and copper 327.4, respectively. Because of increasing self-absorption at higher concentrations, the calibration curve, although initially a straight line, will gradually bend toward the concentration axis.

For a given plot of emission readings against concentration, as in Figure 2, the accuracy attainable at any given luminosity is directly proportional to the slope, $S$, times the concentration at the given point, $G$, and inversely proportional to the minimum difference in concentration, $\Delta G$, that can be detected at that point. The concentration can be expressed as parts per million of copper present.

To determine the optimum value, or range of values, for the luminosity of each copper line, a series of luminosity intervals was studied using varying amounts of copper, and determining when the average value of $(S \times G)/\Delta G$ was a maximum. The flame emissions, after correction for the background radiation, for a typical series of standards are shown in Table I. Assuming that all plots are straight lines over the intervals covered, the slopes are given in columns 3 and 7 for the copper 324.7 and 327.4 m$\mu$ lines, respectively. The slopes continually decrease. The products, $S$ times $G$ (for a median luminosity value) are given in columns 4 and 8, respectively. These rapidly increase to a maximum, then remain fairly constant. The results of the relative accuracy attainable for each line are shown in columns 5 and 9. For purposes of calculation a difference of one scale division (1% luminosity) was assumed to be recognizable.

The second type of calibration curve was obtained by the method of internal standardization. The silver 328.0 m$\mu$ line was chosen as the most ideal emission line for use as internal standard for these reasons:

Silver itself is seldom present in the alloys under examination.
Both the silver and the copper lines are low energy lines which involve similar energy level transitions and which possess similar excitation potentials, the values being 3.80 volts for Cu 324.7, 3.77 volts for Cu 327.4, and 3.75 volts for Ag 328.0.
Both the silver and the copper lines are subject to self-absorption to approximately the same degree.
The silver line is conveniently located relative to the two copper lines, so as to reduce errors to differences in general radiant energy.

Silver salts can be obtained in a very high state of purity with respect to copper and any other elements that might offer serious interference to the flame analysis of copper.

The ratio of the average relative luminosity $(L - H)$ of the copper line to that of the silver line (also $L - H$)—i.e.,

$$\frac{L \text{ of Cu } 324.7 - H \text{ at } 325.0}{L \text{ of Ag } 328.0 - H \text{ at } 328.3}$$

for example—is plotted against concentration of copper on log-log paper to give the calibration curves. The plot of log luminosity ratio $vs.$ log concentration gives a straight line over limited concentration intervals. As might be expected from the variance of the copper luminosities with concentration, a fixed amount of silver serves ideally as internal standard over only a limited range of copper concentrations. The following ranges were found suitable for quantitative work: 50.0 p.p.m. of silver for the interval 10.0 to 100 p.p.m. of copper, and 100 p.p.m. of silver for the interval 75 to 400 p.p.m. of copper.

## Table I.  Selection of Optimum Copper Concentration

| Copper, P.P.M. | For Cu 324.7 Line Emission, scale divisions | $S$ | $S \times G$ | Relative accuracy | For Cu 327.4 Line Emission, scale divisions | $S$ | $S \times G$ | Relative accuracy |
|---|---|---|---|---|---|---|---|---|
| 20 | 29.5 | | | | 18.0 | | | |
| | | 1.05 | 26.3 | 33 | | 0.75 | 18.7 | 14.4 |
| 30 | 41.0 | | | | 25.5 | | | |
| | | 1.00 | 35.0 | 35 | | 0.70 | 24.5 | 17.5 |
| 40 | 51.0 | | | | 32.5 | | | |
| | | 0.85 | 38.2 | 32 | | 0.65 | 29.3 | 19.5 |
| 50 | 59.9 | | | | 39.0 | | | |
| | | 0.75 | 41.2 | 32 | | 0.60 | 33.0 | 19.5 |
| 60 | 67.0 | | | | 45.0 | | | |
| | | 0.60 | 39.0 | 23 | | 0.55 | 35.7 | 19.8 |
| 70 | 73.0 | | | | 50.5 | | | |
| | | 0.55 | 41.2 | 23 | | 0.50 | 37.5 | 18.7 |
| 80 | 78.5 | | | | 55.5 | | | |
| | | 0.50 | 42.5 | 21 | | 0.45 | 38.0 | 17.3 |
| 90 | 83.5 | | | | 60.5 | | | |
| | | 0.45 | 42.7 | 19 | | 0.40 | 38.0 | 15.2 |
| 100 | 88.0 | | | | 64.0 | | | |
| 100 | 59.0 | | | | 41.0 | | | |
| | | 0.280 | 35.0 | 10 | | 0.29 | 35.2 | 10.0 |
| 150 | 73.0 | | | | 55.5 | | | |
| | | 0.160 | 28.0 | 4.5 | | 0.25 | 43.7 | 10.9 |
| 200 | 81.0 | | | | 68.0 | | | |
| | | 0.130 | 29.2 | 3.7 | | 0.19 | 42.8 | 8.2 |
| 250 | .87.5 | | | | 77.5 | | | |
| | | 0.110 | 30.3 | 3.3 | | 0.15 | 41.3 | 6.2 |
| 300 | 93.0 | | | | 85.0 | | | |

In the internal standard method the experimental conditions under which the luminosity ratio is measured cannot usually be perfectly duplicated. If this were not so, a permanent standardization could be made and no further readings of luminosities from standard solutions would be required in a routine series of applications. Unfortunately, as pointed out by Churchill (3), many variables in spectroscopic procedures other than concentration affect the measured luminosity ratios. As a result, working curves show a slight irregular drift and it is necessary to run frequent standard samples to correct for this drift. While all the causes of drift are not understood, it is known that a part of the drift is caused by an actual change in the luminosity ratio and a part is caused by errors in measuring the luminosity ratio. The principal factor affecting the actual luminosity ratio produced by a flame excitation source is the gradual clogging of the burner orifices by carbon deposits, particularly the very constricted oxygen orifice on the metal burners used with the Beckman instrument. In turn this results in a different fuel ratio and a change in the aspiration rate. Both of the latter will affect the flame temperature, and unless the copper and silver are present in exactly equivalent amounts with respect to their luminescence, a variation in the luminosity ratio must be expected.
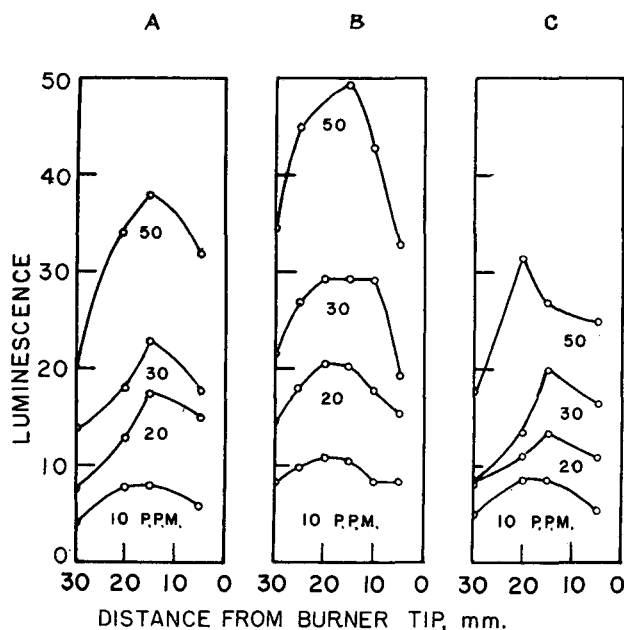


**Figure 4.  Luminescence of copper 324.7 m$\mu$ line as a function of distance from burner tip and for various fuel and oxygen pressures**

*A.* Oxygen pressure, 8 pounds per square inch. Acetylene pressure, 5 pounds per square inch
*B.* Oxygen pressure, 6 pounds per square inch. Acetylene pressure, 5 pounds per square inch
*C.* Oxygen pressure, 6 pounds per square inch. Acetylene pressure, 4 pounds per square inch

### Table II. Influence of Acids

| Acid Concentration, Moles/Liter | Copper Found[a] | |
|---|---|---|
| | 324.7 m$\mu$ | 327.4 m$\mu$ |
| HClO₄ | | |
| 0.5 | 40 | 40 |
| 1.0 | 40 | 40 |
| 1.5 | 41 | 40 |
| 2.5 | 41 | 39 |
| 5.0 | 45 | 42 |
| HCl | | |
| 0.5 | 40 | 40 |
| 1.0 | 40 | 40 |
| 2.5 | 39.5 | 40 |
| 5.0 | 43 | 38 |
| HNO₃ | | |
| 0.5 | 41 | 40 |
| 1.0 | 41 | 40 |
| 1.5 | 41 | 42 |
| 2.5 | | 42 |
| 5.0 | 47 | 43 |
| H₂SO₄ | | |
| 0.5 | 40 | 40 |
| 1.0 | 40 | 39 |
| 1.5 | 38 | 37 |
| 2.5 | 38 | 36 |
| 5.0 | 36 | 35 |

[a] 40.0 p.p.m. of copper present in each solution.

from tin-base alloys. Consequently, tin, along with any antimony and arsenic, was removed from all samples by volatilization of the bromides.

Thus, of all the elements enumerated in Table III, only appreciable amounts of nickel, as might be encountered in nickel alloys, could be construed as offering any serious interference.

### Table III. Influence of Diverse Elements

| Element Tested | Concentration, P.P.M. | Copper, P.P.M. | |
|---|---|---|---|
| | | Present | Found |
| Aluminum | 1,000 | 20 | 20 |
| | 2,000 | 20 | 20 |
| | 5,000 | 20 | 20 |
| Cadmium | 2,000 | 40 | 40 |
| | 4,000 | 40 | 40 |
| Chromium | 1,000 | 20 | 20 |
| | 2,000 | 20 | 21 |
| Cobalt | 1,000 | 20 | 18 |
| | 2,000 | 40 | 34 |
| Iron | 1,000 | 40 | 40 |
| | 2,000 | 40 | 42 |
| Lead | 4,000 | 40 | 40 |
| | 10,000 | 40 | 40 |
| Magnesium | 1,000 | 40 | 41 |
| | 2,000 | 40 | 42 |
| Manganese | 100 | 20 | 20 |
| | 500 | 20 | 20 |
| Nickel | 1,000 | 40 | 40 |
| | 2,000 | 40 | 42 |
| | 4,000 | 40 | 45 |
| Potassium | 2,000 | 40 | 40 |
| | 5,000 | 40 | 40 |
| Sodium | 2,000 | 40 | 40 |
| | 5,000 | 40 | 40 |
| Zinc | 1,000 | 20 | 20 |
| | 3,000 | 20 | 20 |
| | 9,000 | 100 | 100 |

## INFLUENCE OF ACIDS

The influence of hydrogen ion concentration and the effect of various anions commonly associated with the former in acids used for the dissolution of the alloys were considered first. The results are tabulated in Table II. Any of the common acids—perchloric, sulfuric, hydrochloric, or nitric acid—can be tolerated at concentrations 1.0M or less. Obviously, the use of hydrochloric acid is precluded when silver ions are incorporated in the solutions as an internal standard. Acid concentrations exceeding 1.0M generally caused the flame background to increase slightly without any appreciable increase in copper luminosity. Of the two copper lines, the copper 327.4 line proved less susceptible to alteration in intensity when larger amounts of acid were present than did the copper 324.7 line. This was equally true both for "L − H" measurements and for internal standard measurements.

## INFLUENCE OF OTHER ELEMENTS

Of the other elements investigated, only those were considered which constitute the matrix of the copper-containing alloys or were present in these alloys in appreciable quantities. For each substance tested for interference, a series of solutions was prepared containing several known concentrations of the test substance and 40 p.p.m. of copper. Table III shows the effect of various elements on the measurement of copper. The noninterference of large quantities of aluminum, cadmium, lead, and zinc is noteworthy. This enables the flame spectrophotometric method for copper to be applied to aluminum-, lead-, and zinc-base alloys without prior separations if no other elements are present in concentrations which offer interference. The small amounts of nickel, chromium, iron, and magnesium normally encountered in these alloys offer no difficulties. Large amounts of the alkalies can be tolerated, should it be necessary to introduce them.

Brief tests with tin indicated the impossiblity of preventing extensive hydrolysis with the amounts of tin normally present in samples composited

## METHOD OF PROCESSING SAMPLES

**Procedure for Aluminum- and Zinc-Base Alloys.** Weigh samples containing 1 to 15 mg. of copper into 150-ml. beakers. Dissolve in the minimum amount of 6N perchloric acid or 8N nitric acid. Heat to action and simmer until all the sample is dissolved. Raise the cover glasses with hooks and evaporate nearly to dryness to remove excess acid. Add 25 ml. of demineralized water and transfer to a 50-ml. volumetric flask. Add 2.50 ml. of standard silver solution, and dilute to the mark with demineralized water. Mix the solution well. Atomize the solution into the flame, and read the luminosities on the instrument.

Bracket the unknowns with a series of standards. Obtain the luminosities at the following wave lengths: copper at 324.7 m$\mu$, background at 325.0 and 327.0 m$\mu$, copper at 327.4 m$\mu$, silver at 328.0 m$\mu$, and background at 328.3 m$\mu$. Two series of readings, or more if necessary to obtain reasonable duplication, are taken for both standards and unknowns. The appropriate background

### Table IV. Analysis of Bureau of Standards Samples by Flame Spectrophotometric Method

| Sample | Concn. of Sample, P.P.M. | Certified Cu Value, % | Values Found, % | |
|---|---|---|---|---|
| | | | L − H method | Internal standard |
| Aluminum alloy 85a | 1,800 | 2.48 ± 0.01 | 2.52 ± 0.04 | 2.48 ± 0.04 |
| 94 Al, 2 Mg | | | | |
| Aluminum alloy 86c | 5,000 | 7.92 ± 0.03 | 8.11 ± 0.13 | 8.19 ± 0.17 |
| 90 Al, 1 Zn, 1 Fe | 1,250 | | 8.00 ± 0.18 | 8.00 ± 0.20 |
| Aluminum alloy 87 | 20,000 | 0.30 ± 0.01 | 0.297 ± 0.006 | 0.293 ± 0.006 |
| 89 Al, 2 Zn | | | | |
| Tin-base alloy 54b | 2,500 | 3.19 ± 0.02 | 3.24 ± 0.06 | 3.18 ± 0.10 |
| 87 Sn, 7 Sb, 2 Pb | | | | |
| Tin-base alloy 54c | 2,000 | 4.30 ± 0.02 | 4.33 ± 0.08 | 4.34 ± 0.10 |
| 86 Sn, 7 Sb, 2 Pb | | | | |
| Zinc-base alloy 94a | 10,000 | 1.08 ± 0.00₁ | 1.10 ± 0.02 | 1.08 ± 0.03 |
| 95 Zn, 4 Al | 5,000 | | 1.10 ± 0.03 | 1.09 ± 0.04 |

Values given are mean of a series of results, with associated standard deviation, $\sigma = \sqrt{\dfrac{(\Sigma D^2)}{n-1}}$

readings are subtracted from the unknown and standard readings to obtain net relative luminosities, which are averaged for each sample. The average net relative luminosities of the standards are plotted against concentration to give $L - H$ calibration curves for both copper lines to which the average relative luminosities of the unknowns are referred. Or, if the internal standard method of calibration is employed, the ratio of the average net relative luminosities of the copper line to that of the silver line is plotted against concentration of copper in the standards on log-log graph paper to give the respective calibration curves.

**Procedure for Tin-Base Alloys.** Weigh samples containing 1 to 15 mg. of copper into 125-ml. Erlenmeyer flasks. Dissolve in 20 ml. of 48% hydrobromic acid containing 2 ml. of bromine. Cover and heat gently until dissolution of the sample is complete. Add 10 ml. of 12$M$ perchloric acid and heat in a well-ventilated hood, while swirling, over an open flame, until white fumes first appear. Then heat moderately and intermittently to decompose any lead bromide and to expel all hydrobromic acid. A stream of compressed air passed into the flask materially hastens the removal of stannic bromide and antimony bromide. For large amounts of tin, it may be necessary to repeat the volatilization step with an additional 5 ml. of hydrobromic acid.

Treat the entire sample residue, or an aliquot portion, in the same manner as the alloys.

### DISCUSSION

Table IV summarizes the results obtained on Bureau of Standards aluminum-, tin-, and zinc-base alloys. The reproducibility of the flame analyses was very good. On replicate samples the standard deviation from the mean was approximately 3%. In many cases the results obtained were within adequate agreement with the certificate values.

Because of the rapidity with which flame analyses can be accomplished, the procedure for copper offers a competitive method for the determination of copper rivaling the conventional colorimetric or gravimetric methods. Dissolution of the sample is the only preliminary step required prior to the actual flame measurements, which themselves require only a few minutes of the operator's time. The precision of flame analyses is not quite as high as for some colorimetric methods and for most gravimetric methods, but for many purposes the higher degree of precision is not required. It is in this latter type of analyses that

flame spectrophotometric procedures should particularly appeal to analysts.

Results obtained for the samples enumerated in Table IV are apparently unaffected by the measurement method employed. Those obtained by $L - H$ measurements and by the internal standard method show no significant differences. This is interpreted to mean that the measurement of the flame luminosity at the base of the emission lines of copper will adequately compensate for variations in the flame background, and that furthermore, the copper emission lines are essentially unaffected by the presence of relatively large amounts of diverse elements in the flame. Apparently, whenever the flame background is enhanced or depressed by other elements, the copper line emission remains virtually unchanged, and is simply an additive factor onto the flame background. Consequently, at least for the classes of alloys reported in this investigation, it is not necessary to go to the trouble of incorporating an internal standard element in the sample and observing the additional luminosity readings required.

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# Flame Photometric Determination of Lithium in Silicate Rocks

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Wet chemical methods are unsatisfactory for the determination of small amounts of lithium in rocks, so that geochemical studies have depended on spectrographic methods. Trace amounts of lithium in silicate rocks and minerals are rapidly determined by flame photometry. The alkali metals and magnesium are separated from other rock constituents by an acid decomposition followed by a single precipitation with basic lead carbonate. A Beckman DU spectrophotometer is used and the test solution is burned in an inexpensive flame attachment using natural gas and compressed air. Sodium and potassium interferences are compensated for by the use of appropriate additions to the lithium standards. The method is sensitive to 5 p.p.m. of lithia in the original silicate sample, with a maximum deviation of 0.0005% of lithia in the range 0.001 to 0.03%. The method can be applied to a wide variety of silicate materials.

FLAME photometric methods are gradually displacing wet methods for the determination of the alkali metals. The J. Lawrence Smith method, although useful for the determination of sodium and potassium in silicate rocks and minerals, is not suitable for the determination of lithium. Williams and Adams (10) and Broderick and Zack (3) have applied the flame photometer to the determination of lithium in glass; Brumbaugh and Fanus (4) and Beer (2) to the analysis of spodumene; and McCoy and Christiansen (7) to portland cement. None of these methods, or the recent fluorometric method of White, Fletcher, and Parks (9), has been applied to the determination of trace amounts of lithium in silicate rocks.

The determination of trace amounts of lithium in silicate rocks and minerals has been confined to spectrographic methods. In this field, the procedures of Strock (8) and Lundegårdh (6) have been most widely used. The flame spectrophotometer gives results for trace amounts of lithium that compare favorably with determinations made with the spectrograph.

## Table I.   Intensity of Radiation at 671 M$\mu$ for Different Concentrations of Sodium and Potassium

(0.4-mm. slit; full sensitivity.   At same setting, 1 p.p.m. of Li$_2$O will give a reading of approximately 4.0)

| Na$_2$O or K$_2$O, P.P.M. | Reading on $T$ scale Na$_2$O | Reading on $T$ scale K$_2$O |
|---|---|---|
| 0 (water) | 0 | 0 |
| 120 | 0 | 0.2 |
| 240 | 0.2 | 0.4 |
| 300 | 0.2 | 0.5 |
| 360 | 0.3 | 0.6 |
| 420 | 0.4 | 0.7 |
| 480 | 0.5 | 0.8 |
| 540 | 0.6 | 0.9 |
| 600 | 0.7 | 1.0 |
| 720 | 0.9 | 1.2 |

### APPARATUS

A Beckman Model DU spectrophotometer was used with a flame attachment employing an air–natural gas flame. The atomizer is similar to that of Barnes and others (1), consisting of hypodermic needles for air and sample solution inlet, rigidly mounted on a stainless steel plate. This plate is set in a rubber stopper which in turn is placed in the mouth of a 2-liter, round-bottomed flask serving as the spray chamber. The latter has a water-sealed continuous drain at the bottom and an exit side-arm tube near the top. Air and aerosol leaving the flask are mixed with natural gas in a Venturi mixer and proceed to the burner. The latter is a Fisher burner top mounted on a borosilicate glass tube, which is set in a 500-ml. Erlenmeyer flask fitted with a side tube for the introduction of the air-gas mixture. All the air used for combustion passes through the atomizer.

Gas consumption is about 5 cu. feet per hour at a pressure of about 8 inches of water. The air requirement is about 37 cu. feet per hour, supplied at 6 pounds per square inch. Careful control of both air and gas flow, especially the latter, is important. Constancy of gas flow is checked by passing the gas through an orifice flow meter equipped with a water manometer having one leg inclined 3 to 4 degrees from the horizontal. The flow of gas is held to a maximum deviation of less than 0.2%.

The settings of the Beckman DU spectrophotometer are as follows: wave length, 671 m$\mu$; selector switch at 0.1; slit at 0.4 mm.; sensitivity control at counterclockwise limit.

### REAGENTS

**Standard Lithium Sulfate Solution, Approximately 0.1$N$.** Weigh out approximately 8.5 grams of lithium hydroxide monohydrate (purified by recrystallization if necessary), dissolve in 300 ml. of distilled water, and neutralize with a slight excess of 4$N$ sulfuric acid, using methyl red indicator. Boil several minutes, and make just alkaline to methyl red with dilute lithium hydroxide solution. Filter the solution and make up to 2 liters. To standardize, pipet 25-ml. samples into platinum dishes, acidify with a drop of 4$N$ sulfuric acid, and evaporate to dryness. Bring the residue to constant weight by repeated heating to a dull red heat over a low flame. Calculate the normality of the solution from the weight of lithium sulfate found.

**Basic Lead Carbonate.** The quality of the reagent is important. Some brands of analytical reagent grade have been found unsuitable owing to a very high sodium content. Merck's reagent grade was used without purification.

### PROCEDURE

Weigh out a 0.5-gram sample of the very finely ground material and transfer to a 50-ml. platinum dish. Moisten with water and add 0.4 ml. of concentrated sulfuric acid, followed by 15 ml. of hydrofluoric acid and a drop of nitric acid. Heat to a temperature just below boiling by either a hot plate or infrared light until sulfuric acid fumes appear. This will require from 1 to 2 hours. Cool, moisten with 2 ml. of water and 0.1 ml. of sulfuric acid, and again evaporate to fumes. Make a third evaporation with 5 ml. of added water to ensure removal of all the fluoride. Take up the residue in 25 to 30 ml. of water and heat, with occasional stirring, until soluble solids are in solution. Transfer to a 150-ml. beaker and dilute to about 70 ml. At this point solution is essentially complete except for a small residue of zircon, corundum, or other minerals not attacked by the acid decomposition.

Heat to near boiling and add basic lead carbonate, with stirring, until the solution is alkaline to methyl red, keeping the beaker covered as much as possible during this addition. Boil several minutes, rinse down, and stir well. Filter on a 11-cm.

paper (S. & S. No. 595, Whatman No. 1). Wash thoroughly with hot water until the volume of the filtrate and washings is about 125 ml. Concentrate to about 30 ml., cool, and filter through a small paper into a 50-ml. volumetric flask, washing with water. Make up to the mark and mix.

The solution prior to the addition of the lead carbonate will contain all of the aluminum, iron, sodium, potassium, lithium, and magnesium as sulfates, with a little free sulfuric acid. Lead carbonate neutralizes the free sulfuric acid and precipitates iron and aluminum without increasing the salt concentration in the solution. Excess lead carbonate is insoluble, and does not raise the pH much above 6. The precipitate is easily filtered and washed.

The following technique was used in running sample solutions on the photometer. Approximate sodium, potassium, and lithium determinations are made using calibration curves for these elements. This information is used to prepare lithium standards approximately equivalent to the unknown in sodium and potassium concentration, and bracketing the approximate lithium content. The sample is compared with these standards.

### EXPERIMENTAL

**Interferences.** In the determination of trace amounts of lithium in silicate rocks the interference of macro quantities of sodium, potassium, and magnesium must be considered. Continuum and radiation interferences for these three elements were investigated.

A series of standard solutions of sodium and potassium sulfate was prepared from the pure salts. These solutions were run on the instrument at the settings previously given, and the emission at 671 m$\mu$ was measured to obtain the continuum effect as given in Table I. (Magnesium up to 360 p.p.m. showed no effect, and is omitted from the table.) This interference is positive and additive to the lithium emission.

## Table II.   Effect of Radiation Interference

| Sodium and Potassium Content of Sample Solution Na$_2$O, p.p.m. | Sodium and Potassium Content of Sample Solution K$_2$O, p.p.m. | Li$_2$O Found in Rock, % Corrected for continuum interference only | Li$_2$O Found in Rock, % Corrected for continuum and radiation interference |
|---|---|---|---|
| 527 | 300 | 0.0015 | 0.0015 |
| 527 | 300 | 0.0040 | 0.0030 |
| 527 | 300 | 0.0051 | 0.0048 |
| 830 | 537 | 0.0060 | 0.0074 |
| 830 | 537 | 0.0099 | 0.0114 |
| 500 | 330 | 0.0107 | 0.0118 |
| 500 | 330 | 0.0156 | 0.0165 |
| 510 | 360 | 0.0173 | 0.0200 |
| 510 | 360 | 0.0205 | 0.0243 |

The radiation interference effect of sodium and potassium on lithium is negative—that is, the presence of these alkalies depresses the intensity of the lithium emission. This was studied by preparing sample solutions of rocks having a wide range of lithium content, comparing first with pure lithium standards, and then with lithium standards containing sodium and potassium. By bracketing the sample with pure lithium standards and deducting from the gross sample reading the background blank obtained on a solution with sodium and potassium equal to that of the sample solution, a lithium value is obtained which is corrected for continuum interference, but not for radiation interference. Next, by repeating this, but using lithium standards with sodium and potassium concentrations equal to that of the sample solution, the lithium value obtained is corrected for both types of interference. These results are given in Table II.

Investigation of radiation interference of magnesium sulfate showed a negligible effect up to a concentration of 360 p.p.m. of magnesium.

**Table III. Recovery of Additions of Lithia**

| % Li₂O in Rock | % Li₂O Added | Total % Li₂O | % Li₂O Found |
|---|---|---|---|
| 0.0015 | 0.0003 | 0.0018 | 0.0019 |
|  | 0.0003 | 0.0018 | 0.0020 |
|  | 0.0015 | 0.0030 | 0.0030 |
|  | 0.0030 | 0.0045 | 0.0048 |
|  | 0.0058 | 0.0073 | 0.0074 |
|  | 0.0060 | 0.0075 | 0.0065 |
|  | 0.0075 | 0.0090 | 0.0085 |
| 0.0113 | 0.0003 | 0.0116 | 0.0118 |
|  | 0.0021 | 0.0134 | 0.0135 |
|  | 0.0039 | 0.0152 | 0.0149 |
| 0.0111 | 0.0091 | 0.0202 | 0.0200 |
|  | 0.0136 | 0.0247 | 0.0243 |

**Table IV. Comparison of Flame Spectrophotometer and Spectrographic Methods**

|  | % Li₂O | |
|---|---|---|
|  | Flame spectrophotometer | Spectrograph |
| Standard granite G-1 | 0.0052 | 0.004 |
|  | 0.0049 | 0.005 |
| Standard diabase W-1 | 0.0030 | 0.002 |
|  | 0.0033 | 0.002 |
|  | 0.0027 |  |

## PRECISION AND ACCURACY

The sensitivity of the instrument at the settings used (full sensitivity, 0.4-mm. slit, 671 mμ) was 0.05 p.p.m. of lithia in solution, corresponding to 0.0005% of lithia in the sample. A difference of this amount of lithia could usually be detected (see Table III).

Because no standard materials are available for analysis, the only check on the accuracy is by adding known amounts of lithia to the rock powders before decomposition and checking recovery. This was done on rocks on which replicate determinations had been made, the average value being used as the true value. Results of these determinations are shown in Table III.

As an additional check a standard diabase and granite (5) were run several months apart. These results are compared in Table IV with spectrographic results (5) and indicate that the flame spectrophotometer compares favorably with the spectrograph.

Replicate determinations on a variety of samples showed a maximum deviation of 0.0005% of lithia in the sample or 0.05 p.p.m. of lithia in solution in the range 0.05 to 3 p.p.m. of lithia. Average deviation for multiple determinations is about 0.0003% of lithia. The method is considered to be accurate to 0.001% of lithia in the sample.

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# Amperometric Titration of Iron with 1-Nitroso-2-naphthol

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An amperometric titration of iron(III), in acetic acid–sodium acetate buffer, with 1-nitroso-2-naphthol gave satisfactory results. It was the purpose of this investigation to determine optimum conditions for obtaining reproducible and accurate results in the direct titration of iron with this reagent. The titration is more rapid than the gravimetric methods, and necessitates fewer operations than other amperometric methods for the determination of iron. Of the cations studied, only lead interferes. Iron in iron ores was determined rather accurately, after it had been separated by ether extraction. This method should be especially suitable for determination of iron in selected steels.

SANDBERG (4) has performed amperometric titrations of iron(III) with bromoxine (5,7-dibromo-8-hydroxyquinoline) at 50° C. In his work the titration error is reported to be 2% for a 0.1mM concentration of iron, and not more than 0.7% for concentrations of iron greater than 0.4mM. Kolthoff and Liberti (2) have carried out satisfactory amperometric titrations of iron(III)

solutions with cupferron, the ammonium salt of phenylnitrosohydroxylamine, in tartrate and citrate buffers. They recommended that, in the presence of tartrate or citrate, the iron solutions be titrated in a cell covered with a coat of black paint, since iron(III) under these conditions is readily reduced by light to iron(II). These authors report an error of 1% for solutions not less than 1mM in iron, and an error of 2 to 3% for solutions which are 0.5mM in iron.

This investigation was undertaken to ascertain the possibility of obtaining reproducible and sufficiently accurate stoichiometric results in the direct titrations of iron(III) with 1-nitroso-2-naphthol in acetic acid–sodium acetate buffer. The precipitate formed in the reaction between iron and 1-nitroso-2-naphthol, in acetic acid–water medium, has the composition $(C_{10}H_6NO_2)_3Fe$ (7).

## EXPERIMENTAL

**Reagents and Solutions.** The stock solution of iron was prepared by dissolving 27 grams of Baker's analyzed iron(III) chloride hexahydrate in distilled water. This solution was treated with 10 ml. of concentrated hydrochloric acid to prevent hyrolysis

**Table I.  Amperometric Titration of Iron(III) in Acetic Acid–Sodium Acetate Buffer at −0.5 Volt vs. S.C.E.**

| Iron Taken, Millimoles $\times$ 10$^2$ | 1-Nitroso-2-naphthol, Millimoles $\times$ 10$^2$ | Mole Ratio, Fe/Titrant |
|---|---|---|
| 0.197 | 0.59 | 1:3.00 |
| 0.394 | 1.18 | 1:3.00 |
| 0.984 | 2.97 | 1:3.02 |
| 1.968 | 5.89 | 1:2.99 |
| 2.952 | 8.85 | 1:3.00 |
| 3.936 | 11.9 | 1:3.02 |
| 4.919 | 14.8 | 1:3.01 |
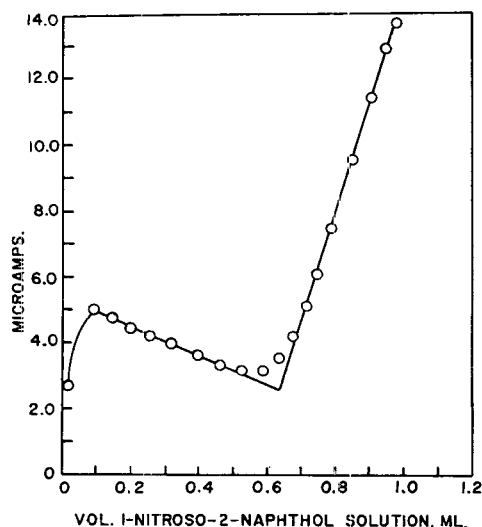


**Figure 1.  Titration of 20 ml. of 0.0984 $\times$ 10$^{-2}M$ iron(III) with 0.0920$M$ 1-nitroso-2-naphthol**

and was then diluted to 1 liter. The stock solution of iron was standardized using potassium permanganate which had been standardized by the sodium oxalate method (5).

1-Nitroso-2-naphthol, Eastman Kodak chemical No. P428, was recrystallized twice from alcohol and dried at room temperature. A weighed amount was dissolved in glacial acetic acid and was then diluted to volume with appropriate volumes of glacial acetic acid and/or distilled water to make the final solution 60% in acetic acid. This solution was filtered to determine whether all the reagent had dissolved, and any small residue remaining was washed and dried at room temperature and the weight of the residue was subtracted from the original weight of the reagent. The concentration of this solution was checked against a standard solution of palladium(II) chloride by the method of Kolthoff and Langer (1); the calculated concentration of 1-nitroso-2-naphthol agreed closely with the concentration determined by the method of Kolthoff and Langer. Fresh solutions of this reagent were prepared every 2 weeks. A buffer solution was prepared which was 2$M$ in acetic acid and in sodium acetate. Water-pumped nitrogen (purity of 99.5% or greater), obtained from the Houston Oxygen, Inc., was used throughout this study. All other materials used were c.p. reagent grade products.

**Apparatus.**  A Sargent Model XII polarograph was employed in this work. The titration cell contained a saturated calomel reference electrode in one compartment, as described by Kolthoff and Lingane (3). All current measurements were taken at 25° $\pm$ 0.1° C. Potentials are expressed versus the saturated calomel electrode (S.C.E.).

On the horizontal portion of the current voltage curve at −0.5 volt, the capillary characteristics for a mercury height of 66.4 cm. were: $m = 1.685$ mg. sec.$^{-1}$, $t = 3.83$ seconds, and $m^{2/3}t^{1/6} = 1.771$ mg.$^{2/3}$ sec.$^{-1/2}$

**Amperometric Titration of Iron with 1-Nitroso-2-naphthol.**  The polarography of iron(III) and 1-nitroso-2-naphthol, in acetic acid–acetate buffer, was investigated briefly for the purpose of determining the shape of their current-voltage curves. These reduction curves, both for iron(III) and for 1-nitroso-2-naphthol,

started at or near zero applied potential and then became essentially parallel with the voltage axis between −0.2 and −1.0 volt.

Preliminary experiments showed that a high concentration of acetate ions was required, and that a large amount of ethyl alcohol was desirable to obtain reproducible results in the amperometric titration of iron(III) with 1-nitroso-2-naphthol; however, the precipitate formed was essentially insoluble in water. The following procedure was adopted for the titration of iron.

An appropriate aliquot of the standard stock solution of iron to give the final concentration desired was added to a 100-ml. volumetric flask, 5 ml. of 0.2% gelatin solution, 20 ml. of 2$M$ acetic acid–acetate buffer, and 20 ml. of 95% ethyl alcohol were added, and the solution was diluted to volume with distilled water. A 20-ml. aliquot of this solution was transferred to an H-type cell, and oxygen-free nitrogen, which had been conditioned by passage through a blank solution that contained all the reagents in the usual concentration except iron or the titrant, was passed through for 15 minutes. After the passage of nitrogen had been completed, a microburet containing the 1-nitroso-2-naphthol solution was pushed through the remaining hole in the stopper of the titration cell. All current measurements were taken at −0.5 volt and were corrected for dilution effects. After the expiration of a few minutes, the current became steady and the mean of the galvanometer deflections was recorded. A small quantity of the titrant was run into the cell; the solution was stirred with nitrogen for 1 minute, and the current was read 3 minutes thereafter. After each addition of titrant, about 2 minutes were required for the current to become constant. This latter procedure was continued throughout the titration. The extrapolation method was employed in ascertaining the volume of titrant used in each titration.

Figure 1 is a typical titration curve for the amperometric titration of iron(III) with 1-nitroso-2-naphthol. The results are shown in Table I; each line in the table is the mean of three closely agreeing results on samples of the concentration indicated.

**Effect of Diverse Ions.**  For application of the method to the analysis of iron ores and common compounds of iron, the possible interference from a number of metal ions should be considered. The metallic ions selected for this study were lead(II), calcium-(II), nickel(II), zinc(II), aluminum(III), titanium(III), and chromium(III). The interference effects of these ions on the amperometric determination of iron(III) were studied by adding equal molar concentrations of the diverse ions, individually, to 1$mM$ solutions of iron (in the final concentration); these solutions were made up in the usual way. The volume of 1-nitroso-2-naphthol required to titrate iron in the presence of equal molar concentrations of lead was approximately 4% greater than the volume of titrant required when lead was not present. Addition of titanium, in the same way, produced a precipitate which settled out almost immediately and did not interfere with the determination of iron. The presence of the remaining diverse ions produced no measurable effect on the end point for the determination of iron.

**Table II.  Determination of Iron in Iron Ores after Ether Extraction**

| Sample No. | Iron, % Amperometric method | Iron, % Gravimetric method | Relative Error, % |
|---|---|---|---|
| 1 | 10.48 | 10.46 | +0.19 |
| 2 | 15.23 | 15.26 | −0.20 |
| 3 | 23.45 | 23.42 | +0.13 |
| 4 | 32.70 | 32.61 | +0.28 |

**Determination of Iron in Iron Ores.**  Weighed samples of four iron ores (obtained from Standard Sample Co., Ames, Iowa) were dissolved using concentrated hydrochloric acid and sodium bisulfate. The iron was extracted from solution using the procedure of Scott (5).

The acid solutions of iron were evaporated to a sirupy consistency and then taken up in dilute hydrochloric acid and transferred to separatory funnels. Iron was extracted four times from

each of the cold acid solutions by shaking with ether; each time the ether which contained the iron was allowed to separate before the lower layer was drawn off for re-extraction. The iron was extracted from the ether by shaking the ether solution with water and drawing off the lower water layer. These extracted iron solutions were transferred to volumetric flasks and the solutions were diluted to volume with distilled water. Aliquots of these solutions were made up according to the standard procedure, duplicate amperometric titrations were performed on each solution, and the iron concentration was determined in the usual manner.

The averages of the results are shown in Table II along with the assays given by the manufacturer for the gravimetric determination of iron in the ores.

## DISCUSSION

The amperometric titration of small amounts of iron, in acetic acid–sodium acetate buffer, with 1-nitroso-2-naphthol has been performed in which satisfactory results were obtained. Of the several diverse ions studied, only lead interfered with the titration of iron; however, iron may be quantitatively extracted from lead using ether (6). Iron in iron ores was determined with a good degree of accuracy, after this element had been separated by ether extraction. The percentage of iron in the ores ranged from 10 to 33%. In no case was the relative percentage error of

a result of this method, as compared with the assay reported by the manufacturer, greater than ±0.28%. Results were duplicated on each of the samples with an average precision of less than ±0.1%. This method as compared with other methods for the amperometric titration of iron(III) (2, 4) saves time and necessitates fewer operations for carrying out the determination.

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# Spectrotitrimetric Determination of Esters in Mixtures

## Benzyl Benzoate and Dibutyl Phthalate

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In a study of cloth impregnations with a mixture of benzyl benzoate and dibutyl phthalate it was necessary to determine the components of this mixture in cloth patches. A method was devised in which the quantity of an alkali used in a saponification and the absorbance reading at 230 mμ were used to determine the quantity of each of these esters. Analyses of five known mixtures showed average recoveries of 100.08% for benzyl benzoate and 99.97% for dibutyl phthalate. Analysis of six impregnated cloth patches showed average recoveries of 101.43% for benzyl benzoate and 99.66% for dibutyl phthalate. The procedure in which titration and spectrophotometric readings are used to analyze for mixtures of the same type of compound is new and has many applications.

A N IMPORTANT phase of research in this laboratory is the investigation of repellents and repellent formulations for use by the military services for the protection of troops against insects, mites, and ticks. One part of this research is the development of procedures for impregnating clothing with repellents. In these procedures it is necessary to have methods of chemical analysis that can be utilized to identify the repellents individually and in mixtures, and to measure the amounts deposited on cloth. Final recommendations as to concentrations of repellents and procedures for impregnation are based largely on the results of analytical determinations of the amounts of repellent deposited on the cloth in experimental trials.

One of the clothing impregnants in use by the army is a mixture which, according to military specification (1), contains 45% each of benzyl benzoate and dibutyl phthalate and 10% of a

nonionic emulsifier. Both esters have a maximum absorption in the lower ultraviolet region, but their peaks are so close, 230 mμ for benzyl benzoate and 225 mμ for dibutyl phthalate, that a method based on readings at these wave lengths would be difficult. Since both esters are easily saponified, a method using the saponification and a spectrophotometric reading has been devised.

The emulsifier used in these experiments does not have an ultraviolet peak that interferes with the spectrophotometric reading. It reacts slightly with alkali but not sufficiently to interfere with the analysis. In the analysis of cloth extracts an electrometric Titrimeter is used, since the extracted dye interferes with the reading of a colorimetric end point. In the analysis of emulsifiable concentrates, either an internal indicator or an electrometric Titrimeter may be used.

The calculations are similar to those used in the spectrophotometric determination of multicomponent systems (2).

### APPARATUS AND REAGENTS

The absorbance measurements are made in matched 1-cm. silica cells with a Beckman Model DU spectrophotometer.

All titrations are performed with a Fisher potentiometric Titrimeter.

The alkalimetry titrations are made with approximately 0.1N standard sulfuric acid.

Approximately 0.1N standard ethanolic sodium hydroxide is used for the saponification of the esters. It is prepared by dissolving 4.0 grams of sodium hydroxide in 10 ml. of water, diluting to 1 liter with 95% ethyl alcohol, and titrating with the standard acid.

Ethyl alcohol, 95%, is used as the solvent in the spectrophotometric determinations. When ethyl alcohol from the same lot is used for dilutions and as a reference in the blank cell of the spectrophotometer, further purification is not necessary.

## Table I. Differences in Determination of Benzyl Benzoate and Dibutyl Phthalate from 0.5-Gram Samples

(Caused by errors reading spectrophotometer or Titrimeter)

| Reading Error | Benzyl Benzoate Found, gram | Benzyl Benzoate Error, % | Dibutyl Phthalate Found, gram | Dibutyl Phthalate Error, % |
|---|---|---|---|---|
| Absorbance | | | | |
| 0.003 high | 0.5053 | 1.06 | 0.4966 | 0.68 |
| low | 0.4929 | 1.42 | 0.5047 | 0.94 |
| Buret | | | | |
| 0.3 ml. high | 0.4942 | 1.16 | 0.5122 | 2.44 |
| low | 0.5043 | 0.86 | 0.4890 | 2.20 |

### ANALYSIS OF MIXTURES

Weigh accurately about 1 gram of the repellent mixture into a 100-ml. volumetric flask and make up to volume with 95% ethyl alcohol. Take a 10-ml. aliquot and bring to 100 ml. in the same manner; repeat twice, so that the final solution has a concentration of approximately 0.01 gram of the sample per liter. Read the absorbance in 1-cm. cells in the spectrophotometer at 230 m$\mu$ at a slit width of 0.7 mm.

Transfer a 50-ml. aliquot of the original 100-ml. solution of the sample to a 250-ml. standard-taper Erlenmeyer flask and add 50 ml., accurately measured, of 0.1$N$ ethanolic sodium hydroxide. About twice the amount of sodium hydroxide theoretically required should be present to ensure complete reaction. Reflux gently for 1 hour, when the saponification should be complete. Transfer the contents of the flask to a 400-ml. beaker, rinse twice with small volumes of distilled water, and titrate the excess sodium hydroxide with standard 0.1$N$ sulfuric acid solution, using a potentiometric Titrimeter.

### ANALYSIS OF REPELLENT ON CLOTH

Cut a 0.5-square-foot patch from the garment that has been treated with the repellent mixture and place in the tube of a Soxhlet extractor. Place about 150 ml. of 95% ethyl alcohol in the flask and extract for 3 hours. Cool and transfer to a 200-ml. volumetric flask, rinse twice with small volumes of ethyl alcohol, and bring to volume.

Take a 100-ml. aliquot, add 50 ml. of 0.10$N$ ethanolic sodium hydroxide, and reflux gently for 1 hour to saponify the esters. Transfer to a 400-ml. beaker and titrate with standard sulfuric acid.

Transfer a 10-ml. aliquot of the original extract to a 100-ml. volumetric flask and bring to volume with 95% ethyl alcohol. Dilute to obtain an absorbance reading in the range of 0.300 to 0.500.

### CALCULATIONS

$$M = m_x g'_x + m_y g'_y \tag{1}$$

$$A = a_x g''_x + a_y g''_y \tag{2}$$

where $x$ is benzyl benzoate, $y$ is dibutyl phthalate, $M$ is milliliters of 0.1$N$ sodium hydroxide consumed by the aliquot, $m$ is milliliters of 0.1$N$ sodium hydroxide required to react with 1 gram of the component, $g'$ is grams in aliquot, $A$ is absorbance of diluted aliquot at 230 m$\mu$, $a$ is absorptivity (absorbance per grams per liter), and $g''$ is concentration in grams per liter.

Multiply Equation 1 by milliliters of total sample per milliliters of aliquot $= T$, and Equation 2 by liters of sample $\times$ dilution factor $= F$.

Then

$$TM = m_x g_x + m_y g_y \tag{3}$$

$$FA = a_x g_x + a_y g_y \tag{4}$$

where $g$ is grams of component in sample.
Solve these two equations for the two unknowns $g_x$ and $g_y$.

$$g_x = \frac{m_y FA - a_y TM}{m_y a_x - m_x a_y} \tag{5}$$

$$g_y = \frac{a_x TM - m_x FA}{m_y a_x - m_x a_y} \tag{6}$$

Constants may vary slightly for different samples of benzyl benzoate and dibutyl phthalate and should be obtained from

samples of highest possible purity. The following constants were obtained in this laboratory:

$$a_x = 67.24$$

$$a_y = 28.87$$

$$m_x = 47.17$$

$$m_y = 71.89$$

Substituting these values the following simple equations are obtained:

$$g_x = 0.02071 \, FA - 0.008315 \, TM \tag{7}$$

$$g_y = 0.01937 \, TM - 0.01358 \, FA \tag{8}$$

**Example Showing Ease of Calculation.** SAMPLE. 100 ml. of solution containing 0.5 gram of each of the esters.
ABSORBANCES. For spectrophotometric reading a 10-ml. aliquot is diluted 1 to 1000, so that the concentration is 0.005 gram per liter for each ester. The theoretical absorbance would be

$$A_x = 67.24 \times 0.005 = 0.336$$

$$A_y = 28.87 \times 0.005 = \underline{0.144}$$

$$A_x + A_y = A = \phantom{000} 0.480$$

SAPONIFICATION. For titration a 50-ml. aliquot is saponified with 50 ml. of 0.1$N$ sodium hydroxide and back-titrated with 0.1$N$ sulfuric acid. The theoretical milliliters of 0.1$N$ sodium hydroxide consumed would be

$$M_x = 47.17 \times 0.25 = 11.79$$

$$M_y = 71.89 \times 0.25 = \underline{17.97}$$

$$M_x + M_y = M = 29.76$$

CALCULATION.

$$T = \frac{100}{50} = 2$$

$$F = 0.1 \times 1,000 = 100$$

Then

$$g_x = (0.02071 \times 100 \times 0.480) - (0.008315 \times 2 \times 29.76)$$
$$0.9941 - 0.4950 = 0.4991$$

$$g_y = (0.1937 \times 2 \times 29.76) - (0.01359 \times 100 \times 0.480)$$
$$1.1529 - 0.6523 = 0.5006$$

## Table II. Results of Analyses of Known Mixtures

| Sample | Benzyl Benzoate Gram Present | Benzyl Benzoate Gram Found | Benzyl Benzoate Recovery, % | Dibutyl Phthalate Gram Present | Dibutyl Phthalate Gram Found | Dibutyl Phthalate Recovery, % |
|---|---|---|---|---|---|---|
| 1 | 0.7412 | 0.7404 | 99.88 | 0.3620 | 0.3621 | 100.03 |
| 2 | 0.5532 | 0.5561 | 100.52 | 0.4805 | 0.4800 | 99.90 |
| 3 | 0.4953 | 0.4944 | 99.82 | 0.5034 | 0.5053 | 100.37 |
| 4 | 0.4298 | 0.4285 | 99.70 | 0.5362 | 0.5374 | 100.23 |
| 5 | 0.3625 | 0.3643 | 100.50 | 0.6778 | 0.6799 | 100.31 |

An idea of the precision may be obtained from the foregoing illustration by assuming errors in the spectrophotometric and titration readings. These theoretical errors are based on the absorbance reading being 0.003 too high or too low and the titration reading of 0.3 ml. too high or too low. The differences due to these assumed errors are shown in Table I.

From these data it can be seen that errors in the spectrophotometric readings affect chiefly the analysis of benzyl benzoate, whereas errors in titration affect chiefly the analysis of dibutyl phthalate. Errors of the magnitude shown in the example above are eliminated by careful work and the running of replicate samples.

### EXPERIMENTAL WORK

Known mixtures of benzyl benzoate and dibutyl phthalate in various ratios from 2 to 1 to 1 to 2 were analyzed by this procedure. The results are shown in Table II.

**Table III. Results of Analyses of Impregnated Cloth Patches**

| Sample | Benzyl Benzoate, Gram | | | Dibutyl Phthalate, Gram | | |
|---|---|---|---|---|---|---|
| | Present | Found | Difference | Present | Found | Difference |
| 1 | 0.725 | 0.736 | 0.011 | 0.136 | 0.134 | 0.002 |
| 2 | 0.351 | 0.358 | 0.007 | 0.170 | 0.175 | 0.005 |
| 3[a] | 0.478 | 0.486 | 0.008 | 0.443 | 0.442 | 0.001 |
| 4[a] | 0.478 | 0.482 | 0.004 | 0.645 | 0.627 | 0.018 |
| 5[a] | 0.224 | 0.224 | 0.000 | 0.655 | 0.654 | 0.001 |
| 6 | 0.147 | 0.155 | 0.008 | 0.546 | 0.544 | 0.002 |

[a] Contained approximately 5% of nonionic emulsifier.

The analysis of these five mixtures showed recoveries of from 99.70 to 100.52% for benzyl benzoate and from 99.90 to 100.37% for dibutyl phthalate.

In another test, known quantities of benzyl benzoate and dibutyl phthalate, and in three cases a nonionic emulsifier were applied to cotton twill patches. These were extracted with ethyl alcohol and the extract analyzed for benzyl benzoate and dibutyl phthalate. The results are given in Table III.

The errors ranged from zero to about 5%, which is well within the efficiency required in studies of impregnation methods.

The emulsifier was present in samples 3, 4, and 5 only, and caused no interference in the analyses.

## CONCLUSIONS

The analytical results obtained in these investigations indicate that the method presented is applicable to the analysis of mixtures of these two compounds with an excellent degree of accuracy and precision. The method is also applicable to cloth-impregnation studies, and should be useful in the analysis of other mixtures of esters or acids that absorb in the ultraviolet region.

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# Detection and Quantitative Determination of Benzo[a]pyrene in American Shale Oil

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**Although American shale oil has been shown to possess carcinogenic properties, no systematic investigation of its carcinogenic constituents has yet been reported. The present paper describes the detection and quantitative determination of one of these constituents, benzo[a]pyrene, in a typical sample of Colorado shale oil. The methods used, distillation followed by chromatographic fractionation and spectrophotometric investigation of the chromatographic fractions, are described and discussed. They permit the accurate and precise determination of benzo[a]pyrene in shale oil. The investigated shale oil sample contained between 0.003 and 0.004% benzo[a]pyrene.**

CRUDE shale oil is a viscous dark brown oil from which liquid fuels and chemicals are obtained by fractional distillation and other refining procedures. The crude shale oil itself is obtained by pyrolysis of oil shale, a solid material found in large deposits in the United States. American shale oil exhibits carcinogenic properties (14, 28), and represents, therefore, a health hazard to workers engaged in its manufacture and processing. Consequently it was of interest to undertake an investigation of its carcinogenic constituents. As a first step in this direction a qualitative and quantitative determination of benzo[a]pyrene in a typical sample of Colorado shale oil was made. The present paper reports the methods used in the shale oil analysis and the results obtained. The crude shale oil is fractionated by vacuum distillation and by a series of chromatographic separations. Fractions are thus obtained that exhibit the absorption and fluorescence spectrum of benzo[a]pyrene. A quantitative evaluation of these spectra permits an accurate and precise determination of the amount of benzo[a]pyrene

present in the original shale oil sample. Bioassays are now under way to determine the possible carcinogenicity of certain benzo[a]pyrene–free shale oil fractions. The results of these assays will be reported later.

## EXPERIMENTAL

**Material and Reagents.** Shale Oil, N-T-U crude shale oil, received from the Bureau of Mines, Laramie, Wyo.

Benzo[a]pyrene, L. Light and Co., Ltd., Colnbrook, Bucks, England, was chromatographed on activated alumina. A cyclohexane-benzene mixture containing up to 80% benzene was used as eluent. The eluate fraction containing the bulk of the benzo[a]pyrene was evaporated under reduced pressure; melting point, 179.0–179.5° C. (corrected).

Cyclohexane, commercial grade, Phillips Petroleum Co.

Benzene, reagent grade.

Dichloromethane, Eastman Kodak.

Methanol, synthetic, 99.9% pure.

All solvents contained impurities that fluoresce in ultraviolet light. They were therefore purified, methanol by refluxing and distilling over freshly precipitated silver oxide, all other solvents by shaking with charcoal (Norit A) (25), followed by distillation. In all cases the first and last 5 to 10% of the distillate were discarded.

Florisil, a magnesium trisilicate, 30- to 60-mesh, air-dried, Floridin Co., Warren, Pa.

Activated alumina, F-20, 80- to 200-mesh, Aluminum Ore Co., St. Louis, Mo., reactivated by heating overnight to 200° C.

Silica gel, Grade 912, 28- to 200-mesh, Davison Chemical Corp., Baltimore, Md., reactivated by heating overnight to 150° C.

Nitrogen, essentially free of oxygen. (Seaford nitrogen of the Air Reduction Co., Baltimore, Md., containing not more than 0.002% oxygen, was used by the author.)

**Instruments.** Refractometer, Abbe-56, Bausch and Lomb Optical Co., Rochester, N. Y.

Spectrophotometer, Cary recording spectrophotometer, Model 11 MS, Applied Physics Corp., Pasadena, Calif.

Spectrograph, Gaertner Scientific Corp., Chicago, Ill., Model L253. This is a wide dispersion quartz prism instrument. Eastman Kodak 103 a-O spectrographic plates (2 × 10 inches) were used.

Microdensitometer, recording microdensitometer, Model 6700-P1, Leeds and Northrup, Philadelphia, Pa.

## ANALYTICAL PROCEDURE

**Shale Oil Fractionation.** The fractionation scheme is shown in Figure 1. The shale oil is distilled in vacuo in an all-glass distilling apparatus. (A 10- to 30-gram sample is conveniently distilled in a small laboratory still. Less shale oil may be used if the distillation is carried out in a microstill.) The vapors pass through a jacketed and heated column. It is preferable to use a column of only a few theoretical plates and a low holdup rather than a more efficient column with considerable holdup and pres-sure drop. Straight-path columns with or without spray catchers (29) were found to be satisfactory. The side arm is connected to the column by an inner seal in order to avoid the creeping over of material. The contents of the still pot are stirred by means of a magnetic stirrer. In order to compensate somewhat for the poor fractionating efficiency of the column, the distillation is carried out at a slow rate (about 2 mg. of distillate per minute per gram of charge). A water pump is used until the bath temperature reaches 90° to 100° C., then is replaced by an oil pump, and the pressure is slowly decreased to 30 to 40 microns. The fraction distilling between 400° and 510° C. (750° to 950° F.) at 760 mm. is collected separately. [The boiling points are extrapolated to 760 mm. by means of a conversion table (24).] When the boiling point reaches ca. 510° C. at 760 mm., distillation is discontinued. The bath temperature should not exceed 250° C. at this point.

The fraction boiling between 400° and 510° C. at 760 mm. is dissolved in 1 to 5 volumes of cyclohexane. The solution or an aliquot part thereof is chromatographed on a Florisil column. (Column dimensions, filling of the column, flow rate, and adsorbent-adsorptive ratio are discussed below.) The chromatogram is developed with cyclohexane. The progress of the chromatographic fractionation is followed by visual observation in daylight and in ultraviolet light as well as by refractive index measurements of the effluent. In ultraviolet light, a pale yellow weakly fluorescent zone surrounded by strongly blue to purple fluorescent zones with diffuse boundaries can be seen traveling down the column. When the advancing blue fluorescent zone approaches the low end of the column, the receiver is changed. After the refractive index of the effluent has reached the same value as the refractive index of the cyclohexane used within ±0.0002 unit, development is continued with methanol, but the receiver is not yet changed. In the course of the development with methanol various zones are observed in daylight: a light purplish gray zone at the top of the column, followed by a light brown zone, then a dark purple or purplish brown line or narrow zone, and finally a brown zone extending to the bottom of the column. As soon as the narrow dark zone is about to leave the column and the color of the effluent changes suddenly from a faint yellow to a dark brown, the receiver is changed.

A preliminary fractionation of the shale oil distillate can also be achieved in an alternative procedure.

A concentrated solution of the distillate in dichlorometh-ane is placed on an activated alumina column and the chromatogram is developed with the same solvent. In ultraviolet light, a yellow weakly fluorescent zone can be seen moving down the column. It is pre-ceded by a narrow blue fluorescent zone and followed by a broad purplish blue fluorescent zone. When the advancing zone approaches the low end of the column, the receiver is changed. After the trailing zone has completely left the column and the refractive index of the effluent has become the same as the one of the dichloromethane used, collection of the eluate is discontinued. (At this point the effluent still exhibits a blue fluorescence.)

The light-yellow cyclohexane eluate from the chromatographic fractionation on Florisil

is concentrated under reduced pressure to a small volume; the light brown eluate from the fractionation on activated alumina is evaporated completely, and the residue is taken up in 1 to 5 volumes of cyclohexane. In either case the cyclohexane solution is then chromatographed on activated alumina. The chromatogram is developed with cyclohexane. The originally colorless effluent becomes yellow to brown and then again colorless. At the same time, the refractive index of the effluent rises and then returns to the original value. Benzene is then used as developer. In ultraviolet light, a narrow pale yellow zone, followed by a broad purplish blue fluorescent zone with diffuse trailing boundary, can be seen moving down the column. When the pale yellow zone is a short distance from the low end of the column, the receiver is changed. After the bulk of the purplish blue fluorescent zone has left the column, leaving behind a still weakly fluorescent column, and after the refractive index of the effluent has become the same as the one of the benzene used, collection of the eluate is discontinued. The residual fluorescence of the column is not indicative of an incomplete elution of benzo[a]pyrene. It is frequently desirable to follow the course of the elution of benzo[a]pyrene spectrophotometrically. In this case the benzene eluate is collected in a number of small fractions. Those fractions which show spectrophotometric evidence of the presence or possible presence of benzo[a]pyrene are then combined and worked up further.

The yellow or orange benzene eluate is evaporated completely under reduced pressure. The residue is taken up in 1 to 5 volumes of cyclohexane. This solution is then chromatographed on activated alumina. The chromatogram is developed with cyclohexane, which elutes some yellow or brown material. After the refractive index of the effluent has become the same as the one of the cyclohexane used, elution is continued with cyclohexane-benzene mixtures of gradually increased benzene content. Several zones can be observed on the column in ultraviolet light. The one containing the bulk of the benzo[a]pyrene is pale yellow and weakly fluorescent. This zone leaves the column in most cases when a solvent mixture containing 40 to 60 volume % benzene is used. In some cases, however, a higher proportion of benzene is required for the complete elution of the benzo[a]pyrene. The rate of elution changes somewhat with each analysis. It depends on the nature of the adsorptives present on the adsorbent-adsorptive ratio used and on other variables. While visual observation of the column is very helpful, it is not sufficient to determine exactly when benzo[a]pyrene begins to leave the column and when its elution is completed. Such a determination can be made only by means of the absorption and/or fluorescence spectra of the various eluate fractions.

**Spectrophotometric Measurements.** The benzo[a]pyrene content of the eluate fractions can be determined quantitatively by comparison of the relative height of the 403 mμ peak in the absorption spectrum of the eluate (Figure 2, curve I) with the
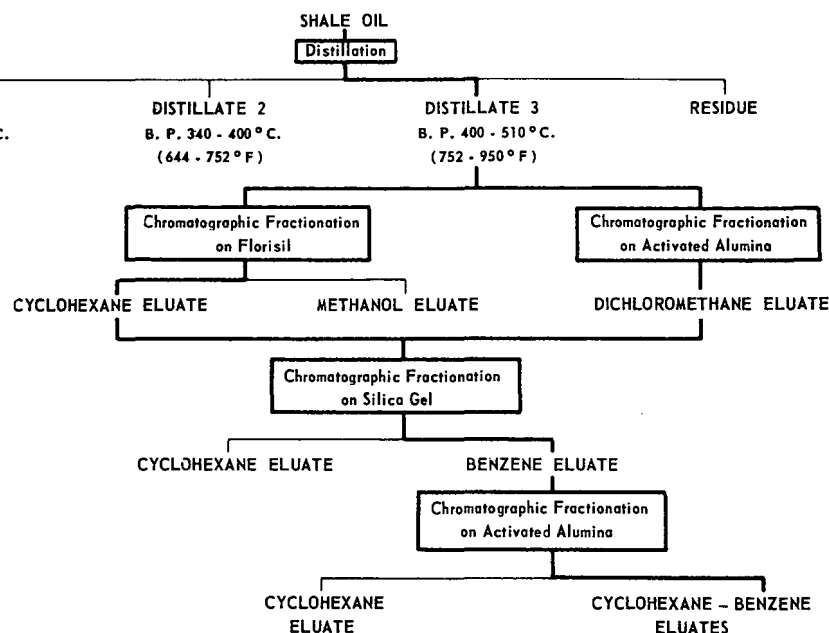


**Figure 1. Shale oil fractionation flow sheet**

relative height of the same peak in the absorption spectra of benzo[a]pyrene solutions of known concentration—e.g., Figure 2, curve V. Although the 403 mμ peak is small, it was chosen rather than the higher 384 and 364 mμ peaks, because background absorption is much less in this region.

In curve I the benzo[a]pyrene spectrum is superimposed on a high background. Many but not all of the substances responsible for this high background absorption can be eliminated by a series of additional chromatographic fractionations (Figure 2, curves II, III, and IV). However, such additional fractionations are not required for the quantitative determination of the benzo[a]-pyrene content of a shale oil sample, as they leave the relative height of the 403 mμ peak unchanged.
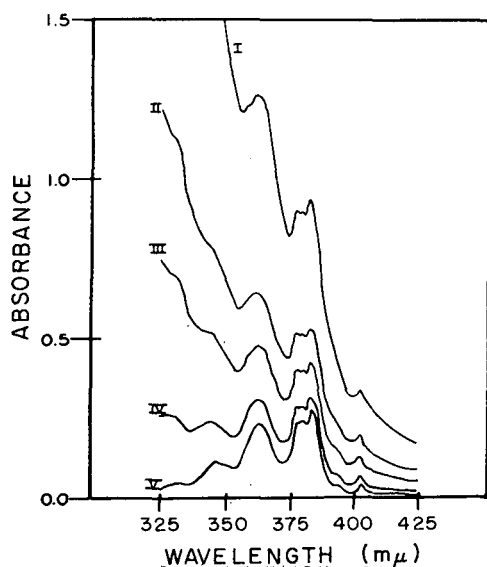


**Figure 2. Absorption spectra of benzo[a]-pyrene and of benzo[a]pyrene–containing eluates obtained in successive chromatographic fractionations**

Solvent. Iso-octane (2,2,4-trimethylpentane), 2-cm. cell
I.  Eluate from chromatographic fractionation on activated alumina, diluted 1 to 20;  84 γ of solute per ml.
II.  Eluate obtained after one additional chromatographic fractionation, diluted to the same volume as I;  43 γ of solute per ml.
III.  Eluate obtained after two additional chromatographic fractionations, diluted to the same volume as I;  25 γ of solute per ml.
IV.  Eluate obtained after three additional chromatographic fractionations, diluted to the same volume as I;  9 γ of solute per ml.
V.  Benzo[a]pyrene, 1 γ per ml.

The fluorescence attachment to the Cary recording spectrophotometer permits the rapid qualitative evaluation of eluate fractions by means of their fluorescence spectra, but not an accurate quantitative determination of benzo[a]pyrene except in pure benzo[a]pyrene solutions. Quantitative determinations can be made by means of a prism spectrograph.

A 1 X 1 cm. stoppered Corex cell is filled with the solution to be analyzed. The cell has four clear sides, one of which is aluminized in order to create a light reflecting mirror. (This mirror which is not indispensable but increases somewhat the sensitivity of the method, was made by the Cumberland Optical Co., Silver Spring, Md.) A fine stream of nitrogen, saturated with the vapor of the solvent used in making up the sample [benzene in most cases (cf. 18)] is bubbled through the solution for 2 minutes in order to eliminate oxygen quenching (34, 35). Then the stoppered cell is placed directly in front of the entrance slit of the spectrograph, so that the side opposite the mirror faces and almost touches the slit. The two adjacent sides are illuminated at an angle of about 90° by the ultraviolet light beams from two General Electric H 100-A4 mercury arc bulbs encased in well venti-

lated metal boxes. The light of the mercury arc passes first through a condensing lens and then through a window in the otherwise lighttight metal box. The window is made of a polished Corning filter No. 5840 (2 square inches, ca. 4.5 mm. thick). The light beams are focused onto the cell in such a manner that the most intense parts of the beams hit those parts of the solution that are closest to the entrance slit of the spectrograph (18). From four to six different spectra are photographed on the same plate together with a wave-length scale. The mercury arc spectrum is superimposed on the scale and serves to calibrate it exactly. All spectra on the same plate are taken at the same temperature and with the sample cell and the two light sources in the same fixed position. A slit width of 50 microns and an exposure time of 1 minute were used in most cases. Somewhat longer exposures and/or larger slits were found to be preferable in some cases. The plates are developed with Eastman Kodak developer D-19 according to the instructions of the manufacturer, or using a slightly longer development time in order to bring out more contrast. By visual comparison of the intensity of the main bands in the spectra of benzo[a]pyrene solutions of known concentration (405, 410, 428, 458 mμ) with the intensity of the same bands in the spectrum of the solution to be analyzed a fairly good evaluation of the benzo[a]pyrene content of the latter can be made. More accurate results are obtained when the band intensities are compared by means of a microdensitometer.
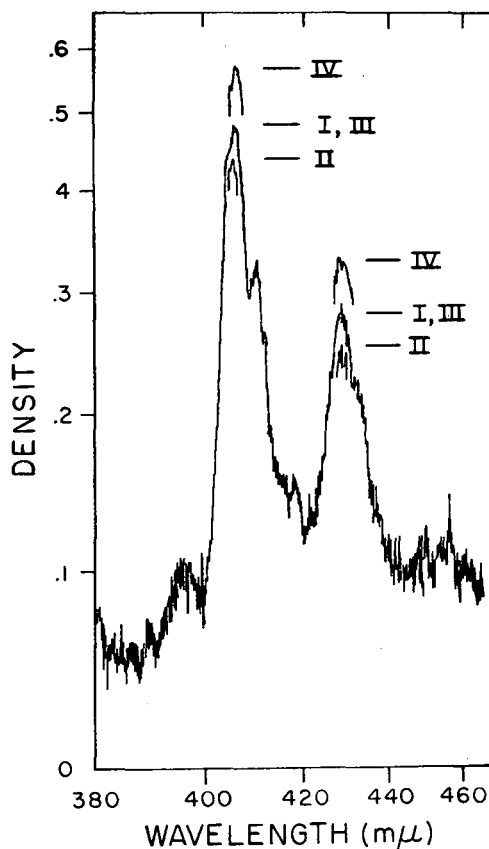


**Figure 3. Fluorescence spectrum of benzo-[a]pyrene–containing eluate obtained after a series of chromatographic fractionations**

Microdensitometer tracing of spectrum obtained with the prism spectrograph
Solvent. benzene
I.  Eluate, diluted 1 to 2000;  0.09 γ of solute per ml.
II.  Benzo[a]pyrene, 0.007 γ per ml.
III.  Benzo[a]pyrene, 0.009 γ per ml.
IV.  Benzo[a]pyrene, 0.011 γ per ml.

Figure 3 shows the microdensitometer tracing of the fluorescence spectrum of an eluate fraction obtained after multiple chromatographic fractionations. The peak regions of the fluorescence spectra of benzo[a]pyrene solutions of known concentration (corrected for slight differences in background fluorescence in these regions) are also shown for comparison.

### Table I. Fractionation of Shale Oil

| Fraction | Amount, Grams | % of Starting Material | Benzo[a]pyrene Concentration, % |
|---|---|---|---|
| Crude shale oil | 840 | 100 | 0.003–0.004 |
| Distillate, b.p. 402–513° C. (755–956° F.) | 218 | 26 | 0.01 |
| Cyclohexane eluate from chromatography on Florisil | 151 | 18 | 0.02 |
| Benzene eluate (first part) from chromatography on silica gel | 66 | 7.9 | 0.04 |
| Cyclohexane-benzene eluate (second part) from chromatography on activated alumina | 2.1 | 0.25 | 1.4 |

## RESULTS

Table I shows the degree of concentration achieved in each step of the fractionation procedure. (A large amount of shale oil was used in this case in order to obtain fractions large enough for bioassays.) One distillation and three chromatographic fractionations reduced the original 840 grams of shale oil to a fraction weighing 2.1 grams or 0.25% of the starting material. At the same time the benzo[a]pyrene content rose from 0.003–0.004% to 1.4%. After three additional chromatographic fractionations (which are not indicated in the table because they are not required for the quantitative determination of the benzo[a]pyrene content of the shale oil) a fraction weighing only 0.24 gram (0.029% of the starting material) was obtained and the benzo[a]-pyrene concentration rose to 12%. A benzo[a]pyrene content of $3.6 \pm 0.7 \times 10^{-3}\%$ is calculated by means of absorption spectrophotometry and one of $3.3 \pm 0.4 \times 10^{-3}\%$ by means of fluorescence spectrophotometry. The two results are in excellent agreement.

## DISCUSSION

The carcinogenicity of Scottish shale oil was demonstrated experimentally as early as 1922 (22). Berenblum and Schoental (1) detected benzo[a]pyrene in a highly carcinogenic fraction technically obtained during the refining of Scottish shale oil. The carcinogenicity of American shale oil was demonstrated much later (14, 23) and no systematic investigation of its carcinogenic constituents has been reported. The presence of benzo[a]pyrene in American shale oil could be suspected, not only because benzo[a]pyrene had been found earlier in a concentrate obtained from Scottish shale oil but also because it is almost invariably found in pyrogenic organic materials of this type, such as coal tar or soot. Oil shale is not carcinogenic (1, 14). Carcinogenicity arises only when the oil shale is subjected to high temperatures in the retorting process that yields shale oil. Similarly, the carcinogenic constituents of tars and soot, among which benzo[a]pyrene is always found, are produced in retorting processes from noncarcinogenic materials.

Minute amounts of benzo[a]pyrene can be determined spectrophotometrically. In pure benzo[a]pyrene solutions less than $10^{-2}$ γ per ml. can be detected by means of absorption spectrophotometry and less than $10^{-3}$ γ per ml. by means of fluorescence spectrophotometry. However, in crude shale oil containing 30 to 40 γ of benzo[a]pyrene per ml. the other shale oil constituents, amounting to about 30,000 times the weight of the benzo[a]-pyrene present, cause so much background absorption as well as background fluorescence and fluorescence quenching that even the qualitative detection of benzo[a]pyrene becomes impossible. The major part of these interfering substances must be eliminated before the benzo[a]pyrene spectrum can be detected. Further purification is required before an accurate quantitative evaluation can be made.

Distillation of the crude shale oil was chosen as a first step in the fractionation procedure because it permits the elimination of 65 to 75% of the interfering substances in a single operation. It is known that a much more efficient resolution can be achieved
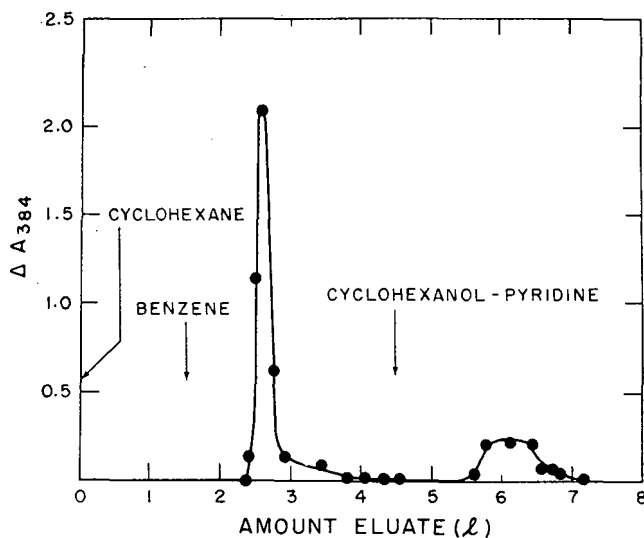


Figure 4. Elution of material absorbing light selectively at about 384 mμ

Chromatographic fractionation on silica gel
$\Delta A_{384}$ = Total absorbance — background absorbance at 384 mμ (calculated for dilution 1 to 100 and light path of 1 cm.)

in the chromatographic fractionation of complex mixtures when the chromatography is performed on distillates rather than on the crude mixture itself (32). The still pot temperature was kept below 250° C. in order to avoid any pyrolytic formation of benzo[a]pyrene. Kennaway (15–17) studied the temperature dependence of the pyrolytic formation of polynuclear aromatic hydrocarbons. From his investigations as well as from those of other investigators (8, 9) it can be concluded that no pyrolytic formation of polynuclear aromatic hydrocarbons takes place at 250° C. With a straight-path still and a jacketed and heated column, the still pot temperature is kept sufficiently low without difficulty. The distilling fraction boiling between 400° and 510° C. at 760 mm. was expected to contain the entire benzo[a]-pyrene of the original shale oil sample, as the boiling point of benzo[a]pyrene lies within this boiling range (2, 10). Nevertheless, since the elimination curve of polynuclear aromatic hydrocarbons is a rather broad one in this type of distillation (10), the fraction boiling between 340° and 400° C. at 760 mm., as well as the distilling residue, were also analyzed for benzo[a]-pyrene in the same manner as the 400° to 510° fraction. A number of polynuclear aromatic hydrocarbons (anthracene, pyrene, anthanthrene, etc.) were detected in these fractions but no benzo[a]pyrene.

Florisil has been recommended (27) for the group separation of shale oil distillates into two fractions, one containing primarily hydrocarbons and the other principally nitrogen compounds. A study of the chromatographic behavior of benzo[a]pyrene showed that it is only incompletely eluted from Florisil by n-pentane or cyclohexane. In order to recover the adsorbed benzo[a]pyrene completely, it was necessary to displace it with methanol as described in the experimental part. In the alternative procedure for the preliminary fractionation of the shale oil distillate—adsorption on activated alumina and elution with dichloromethane—the entire benzo[a]pyrene is eluted rapidly but a darker colored eluate is obtained.

The chromatographic behavior of benzo[a]pyrene on activated alumina and silica gel columns was studied by Weil-Malherbe (36). From his investigations it can be concluded that both adsorbents must be excellently suited for the quantitative chromatographic separation of benzo[a]pyrene. Only one of these adsorbents, activated alumina, has been used by other investigators (1, 3–6, 12, 20, 21, 30, 31, 33) for the quantitative or semi-

quantitative estimation of benzo[a]pyrene in such complex mixtures as tars, soot, and engine exhausts. In the present investigation successive chromatographic fractionations on both adsorbents were carried out. A single fractionation on one of them proved to be insufficient for the accurate quantitative determination of the benzo[a]pyrene content of shale oil and repeated fractionations on either activated alumina or silica gel are less efficient than successive fractionations on both adsorbents.

The absorbent-adsorptive ratio in the various chromatographic fractionations varied from 15 to 30. Considerable changes in the column dimensions do not appreciably alter the column efficiency. Height to diameter ratios varying from about 10 to 50 were used. Low ratios frequently result in too high flow rates unless the flow is regulated with a stopcock. It is preferable to use relatively high ratios and to apply pressure (nitrogen) in order to keep the flow rate at the desired level of about 5 to 15 mm. per minute (cf. 7, 13, 23). Proper packing of the columns is important. Florisil and activated alumina columns are prepared by either dry- or wet-filling. Silica gel columns are preferably prepared by wet-filling because crack formation due to the considerable heat of wetting is thus avoided. All chromatographic fractionations were carried out in subdued artificial light (General Electric fluorescent lamps Gold). Partial decomposition of some polynuclear aromatic hydrocarbons on chromatographic columns under the influence of bright daylight has been observed (11, cf. also 25, 26, 35). The columns were irradiated only momentarily with ultraviolet light for the inspection of fluorescent zones.

The absorption spectra of the benzo[a]pyrene–containing eluates obtained in the first chromatographic fractionation (Florisil or activated alumina) of the shale oil distillate were atypical. In some of the benzene eluate fractions obtained in the second chromatographic fractionation (silica gel) the main bands of the fluorescence spectrum of benzo [a]pyrene, superimposed on a high background fluorescence, could be detected with the spectrograph. The absorption spectra of the same eluate fractions showed a hump at about 384 m$\mu$ and some of them also a smaller hump at about 364 m$\mu$. Figure 4 shows the elution curve of material absorbing light selectively in the region of 384 m$\mu$. Inspection of the curve permits the selection of the eluate fractions possibly containing benzo[a]pyrene. From the shape of the curve it can also be seen that replacement of benzene with a highly polar eluant (cyclohexanol-pyridine) leads to the elution of additional material absorbing light selectively around 384 m$\mu$. The fluorescence spectrum of the cyclohexanol-pyridine eluate was not conclusive and it seemed possible that this eluate contained some additional benzo[a]pyrene not previously eluted with benzene. Further chromatographic fractionation on activated alumina showed, however, that the cyclohexanol-pyridine eluate contained no benzo[a]pyrene but another polynuclear aromatic compound not yet identified.

An accurate quantitative determination of the benzo[a]pyrene content of shale oil became possible after the third chromatographic fractionation (activated alumina) of the shale oil distillate The precision of the absorption spectrophotometric determination was hardly improved by a series of further chromatographic fractionations. The high degree of sensitivity and precision of the fluorescence spectrophotometric method, when applied to highly purified benzo[a]pyrene fractions from shale oil, becomes evident from an inspection of Figure 3. In less pure fractions, still containing a large amount of shale oil constituents other than benzo[a]pyrene, both sensitivity and precision are diminished. The contribution of the background fluorescence to the total fluorescence as well as quenching effects then become important and correction for them is therefore difficult. Thus, no precise comparison of the fluorescence spectrum of the eluate that gave the absorption spectrum shown in Figure 2, curve I, with the fluorescence spectra of benzo[a]pyrene solutions of known concentration could be made. However, when the fluorescence spectrum of the eluate was compared with the spectra obtained from the same eluate after the addition of small known increments of benzo[a]pyrene, the benzo[a]pyrene content of the eluate could be determined with good precision by extrapolation.

Fluorescence and absorption spectrophotometric methods supplement each other. For the accurate and precise determination of benzo[a]pyrene in complex mixtures it is advisable to use one method as a check on the other. [An absorption spectrophotometric method based on difference spectra has been reported since this paper was submitted for publication (30).]

In order to determine the amount of benzo[a]pyrene lost, either mechanically or through destruction, in the course of the shale oil fractionation, a large amount of benzo[a]pyrene (100 mg.) was added to 25 grams of shale oil containing less than 1 mg. of benzo[a]pyrene, and the mixture was then worked up according to the described analytical procedure. The recovery of benzo[a]-pyrene in each step was determined spectrophotometrically. In the distillation step 94% of the added benzo[a]pyrene was recovered in the fraction boiling between 400° and 510° C. at 760 mm. The recoveries in the various chromatographic fractionations varied between 97 and 100%. The over-all recovery after one distillation and three chromatographic fractionations was 89 ± 2%. Recovery experiments were also carried out with such small amounts of benzo[a]pyrene as are actually present in shale oil. When these small amounts of benzo[a]pyrene were passed through columns of various adsorbents under conditions resembling those in the actual shale oil analysis, again 97 to 100% of the added benzo[a]pyrene was recovered.

A comparison of the amount of 0.003 to 0.004% benzo[a]pyrene found in a typical sample of American shale oil with the amount of benzo[a]pyrene found in other complex mixtures is instructive and a few figures are therefore given: About 0.3 to 0.8% of benzo[a]pyrene was found in coal tar (19) and ca. 0.03% in soot (12). Berenblum and Schoental (1) estimated the benzo-[a]pyrene content of a concentrate obtained from crude Scottish shale oil to be of the order of 0.01%, but did not indicate the weight relationship of the concentrate used to crude oil. About 0.0003% benzo[a]pyrene was found in the dried sewage humus that separates from industrial effluents after treatment of the crude sewage in sewage works (33). Cooper, Lindsey, and Waller (6) found in the tar obtained in a smoking machine from 100 cigarettes (110 grams) an amount of benzo[a]pyrene of the order of 1 γ. If it is assumed that 100 cigarettes yielded ca. 4.8 to 4.9 grams of tar (37), the benzo[a]pyrene content of the tar can be calculated to be of the order of 0.00002%.

The analytical method described in this paper has been applied to shale oil only, but there can be little doubt that it is applicable as well to similar complex mixtures such as extracts of air and water pollutants, tars, and high boiling petroleum fractions.

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# Nature and Elimination of Interferences in the Determination of Cobalt with Nitroso-R Salt

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Following an investigation of the nature of 21 elements, a procedure was developed which makes possible a simple and rapid photometric determination of cobalt in a wide variety of substances without the necessity of employing cumbersome procedures for the removal of interfering elements. Tables list the deviation from the standard cobalt value for each of the 21 elements. Those ions that interfere in the cobalt–nitroso-R salt procedure are iron(II), iron(III), cerium(III), chromium-(VI), nickel(II), copper(II), tin(II), and vanadate. Four modified procedures are described. Alkali fluorides used as the complexing agent remedied all the interferences. The results of this investigation and the techniques employed for elimination of interferences may be applicable to other colorimetric procedures.

NITROSO-R salt as an analytical reagent for cobalt was first proposed by Van Klooster (*10*) in 1921. Since then, there have been numerous reports dealing with the elimination of interferences in specific samples. Although some of these investigators have recognized and removed interfering ions, none have attempted to study the mechanism of such interferences as cerium, chromium, copper, vanadium, tin, manganese, nickel, and iron which form complexes with nitroso-R salt that may cause inaccuracies in the determination of cobalt (*3, 5, 13*). Some authors recommend the removal of these and other elements by ion exchange, electrolysis, or precipitation (*2, 4, 6, 11*). Other investigators complex iron with phosphoric acid and remove Group II elements with hydrogen sulfide (*3, 4, 13*). In the determination of cobalt in aluminum alloys, Stross (*9*) compensates the effect of interfering elements by use of a calibration curve prepared with cobalt-free alloys. This procedure is valid for samples of similar composition.

An investigation of the nature and elimination of the interferences of numerous elements has made possible a simple and rapid photometric determination of cobalt in a wide variety of substances without the necessity of employing cumbersome procedures in order to remove interfering cations. Iron(II) and iron(III) have received a more extensive study because they are most commonly associated with cobalt(II) and because their behavior may serve as a prototype for other interferences.

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## REAGENTS, STOCK SOLUTIONS, AND APPARATUS

Sodium Acetate, C.P., 33%.
Hydrochloric Acid, concentrated, C.P., specific gravity = 1.187.
Nitric Acid, concentrated, C.P., specific gravity = 1.410.
Nitroso-R Salt, 0.5 gram per 100 ml.
Potassium Fluoride, 33%.
Cobalt Chloride Hexahydrate, dissolved in distilled water and aliquoted to contain approximately 40 $\gamma$ of cobalt per milliliter.
Chlorides of antimony, cadmium, cerium(II), chromium(III), manganese(II), nickel(II), tin(II), tin(III), and zinc.
Nitrates of bismuth, mercury(II), and uranium.
Sulfates of cerium(III), copper, and vanadyl.
Potassium Dichromate.
Lead Acetate.
Iron Wire, dissolved in concentrated hydrochloric acid and the resulting solution diluted and aliquoted to contain approximately 200 $\gamma$ of iron per milliliter. Solutions containing 10 and 100 $\gamma$ were prepared by dilution with distilled water. These iron solutions were tested with oxidizing or reducing agents to guarantee the valence state prior to use.
Electrophotometer (Fisher) with a 525-m$\mu$ filter.

## EXPERIMENTAL METHOD

Samples containing approximately 40 $\gamma$ of cobalt were treated with varying amounts of the interfering elements. The solutions were buffered to a pH of 5.5 with sodium acetate and acetic acid and a measured amount, according to the intent of the experiment, of nitroso-R salt was added. The solution was heated to boiling, 1 ml. of concentrated nitric acid was added, and the resulting solution was diluted to 25 ml. after it had cooled. The absorbance of the solution was then measured against an appropriate blank with an electrophotometer employing a 525-m$\mu$ filter.

Interfering ions were studied by four modifications of the above procedure:

Potassium fluoride complexation. Addition of potassium fluoride prior to addition of nitroso-R salt.

Acetate complexation. Heating the buffered solution prior to the addition of nitroso-R salt.

Recycling. Alternate acidification with hydrochloric acid and neutralization with sodium acetate after initial color development.

Addition of excess nitroso-R salt.

## EXPERIMENTAL RESULTS AND DISCUSSION

Table I shows the percentage deviation from the standard cobalt value caused by 3000 $\gamma$ of the cations which form no visible complex with the nitroso-R salt.

Table II shows the approximate minimum concentrations of interfering cations that will cause a deviation from the standard cobalt value.

Table III shows the percentage deviation from the normal cobalt value caused by each of the four modified procedures in the presence of the interfering cations.

Iron, cerium, chromium, nickel, copper, vanadium, and tin interfere with the cobalt–nitroso-R salt reaction by several different mechanisms.

**Interference of Iron.** The interference of iron with this reaction has been confirmed by many previous investigators (3–5, 13), most of whom described methods for its removal by precipitation, ether extraction, or complex formation without speculating as to the exact nature of the interference. Scherbov (8) and Dean (2) postulated that iron and cobalt compete for nitroso-R salt in such a way that if there is insufficient reagent to complex all metal ions present some cobalt(II) will remain uncomplexed, resulting in a depression of its absorbance. Scherbov (8) suggested that a three- to fourfold excess or reagent was sufficient to nullify the effect of iron(III).

The present investigation proved that the depression in absorbance of cobalt–nitroso-R salt by iron(III) cannot be remedied by the addition of excess nitroso-R salt. Furthermore, large quantities of nitroso-R salt affect the precision of the cobalt determinations by producing an impractically large reagent blank, particularly if a wave length of 420 m$\mu$ is used in the measurement of absorbance. Marston and Dewey (5) decolorized the excess nitroso-R salt by the addition of bromine after the complete color development, but Sandell (7) states that this is unnecessary when a photometer is used. Experiments performed have shown that under normal conditions, destruction of nitroso-R salt is unnecessary providing photometric measurements are made with a 525-m$\mu$ filter; however, in cases where large quanti-

**Table IV. Effect of Iron(II) and Iron(III) upon Absorbance of Cobalt–Nitroso-R Salt[a]**

| Interfering Ions, $\gamma$ | Absorbance $\times$ 100 | |
| --- | --- | --- |
| | Fe$^{++}$ | Fe$^{+++}$ |
| 20 | 64.9 | 65.0 |
| 40 | 64.9 | 64.9 |
| 60 | 64.8 | 58.4 |
| 100 | 64.5 | 58.0 |
| 200 | 65.0 | 58.0 |
| 300 | 65.0 | 59.0 |
| 350 | 64.0 | .. |
| 375 | 64.2 | .. |
| 400 | 52.0 | 57.0 |
| 1000 | 51.5 | 51.0 |

[a] Concentrations used. 40 $\gamma$ of cobalt per 25 ml., 5 mg. of nitroso-R salt per 25 ml., 0.66 gram of sodium acetate per 25 ml.

**Table V. Effect of Dilution upon Absorbance of Iron(III)–Nitroso-R Salt**

| | Absorbance $\times$ 100 | |
| --- | --- | --- |
| Fe$^{+++}$, $\gamma$ | 1 Ml. nitroso-R salt[a] | 2 Ml. nitroso-R salt |
| 20 | 13.4 | 12.4 |
| 40 | 27.6 | 25.0 |
| 60 | 38.4 | 36.3 |
| 80 | 38.4 | 43.1 |
| 100 | 41.4 | 61.2 |
| 200 | 58.3 | 108.0 |

[a] 1 ml. of nitroso-R salt added before dilution to 25 ml. and 1 ml. added after dilution.

**Table VI. Effect of Temperature upon Depression of Absorbance of Cobalt–Nitroso-R Salt by Iron(III)[a]**

| | Absorbance $\times$ 100 | |
| --- | --- | --- |
| Fe$^{+++}$, $\gamma$ | Room temperature | Boiling temperature |
| 40 | 64.9 | 65.0 |
| 100 | 58.0 | 64.5 |
| 200 | 58.0 | 64.5 |
| 300 | 59.0 | 64.5 |
| 400 | 57.0 | 64.0 |
| 1150 | 51.0 | 73.0[b] |

[a] Concentrations. 40 $\gamma$ of cobalt per 25 ml., 5 mg. of nitroso-R salt per 25 ml., 0.66 gram of sodium acetate per 25 ml.
[b] Higher reading due to color of iron(III) acetate.

**Table I. Effect of Noncomplexed Ions upon Cobalt–Nitroso-R Salt[a]**

| Cation | Deviation from Standard, % | Cation | Deviation from Standard, % |
| --- | --- | --- | --- |
| Sb$^{+++}$ | 0.3 | Zn$^{++}$ | 0 |
| Bi$^{+++}$ | 1.2 | Ce$^{+++}$ | 0 |
| Cd$^{++}$ | 1.2 | Mn$^{++}$ | 0 |
| Pb$^{++}$ | 0 | Mo$^{++++++}$ | 0 |
| Hg$^{++}$ | 0 | UO$_2^{++}$ | 0 |
| Sn$^{++++}$ | 0 | VO$_3^+$ | 0.7 |

[a] Concentrations. 40 $\gamma$ of cobalt per 25 ml., 3000 $\gamma$ of cation per 25 ml., 0.66 gram of sodium acetate per 25 ml.

**Table II. Limit of Interference[a]**

| Cations | Amount, $\gamma$ | Cations | Amount, $\gamma$ |
| --- | --- | --- | --- |
| Fe$^{++}$ | 400 | Ni$^{++}$ | 2400 |
| Fe$^{+++}$ | 60 | Sn$^{++}$ | 1000[b] |
| Ce$^{++++}$ | 100 | VO$^{++}$ | 1000[b] |
| Cr$^{+++}$ | 400 | Cu$^{++}$ | 300–1000[c] |
| Cr$^{++++++}$ | 40 | | |

[a] Concentrations. 40 $\gamma$ of cobalt per 25 ml., 0.66 gram of sodium acetate per 25 ml.
[b] Color fades upon heating because of the reduction of cobalt(III).
[c] Depends upon the concentration of copper(II) when nitroso-R salt is added.

**Table III. Effect of the Modified Procedures[a]**

| Cations | Amount, $\gamma$ | Standard Procedure, % Deviation | KF Complexation, % Deviation | Acetate Complexation, % Deviation | Recycling, % Deviation | Excess Nitroso-R Salt, % Deviation |
| --- | --- | --- | --- | --- | --- | --- |
| Fe$^{++}$ | 400 | −20.0 | 0 | −9.35 | 0 | 0 |
| Fe$^{+++}$ | 400 | −10.6 | 0 | 0 | −10.6 | −16.9 |
| Ce$^{++++}$ | 1000 | −13.9 | −0.75 | −1.4 | −14.3 | −10.1 |
| Cr$^{+++}$ | 3000 | +6.8 | 0 | .... | .... | .... |
| Cr$^{++++++}$ | 1000 | − 7.6 | 0 | +1.5 | −10.8 | −7.6 |
| Ni$^{++}$ | 4000 | −31 | 0 | −28.6 | −48.6 | −16.3 |
| Cu$^{++}$ | 1000 | −15.7 | −0.85 | −7.15 | −18.3 | −10.0 |

[a] Concentrations. 40 $\gamma$ of cobalt per 25 ml., 0.66 gram of sodium acetate per 25 ml., 0.33 gram of potassium fluoride per 25 ml. These concentrations are not present in every case, but are the concentration used when added.

ties of nitroso-R salt must be employed the excess of reagent must be destroyed. Willard and Kaufman (12) studied the absorption spectrum of cobalt–nitroso-R salt and concluded that 420 m$\mu$ was the optimum wave length. Other investigators (1, 6, 7) have preferred to make measurements at 510, 525, or 550 m$\mu$. Most of these procedures were developed primarily for the determination of very small quantities of cobalt in the presence of large quantities of interfering ions.

EXPERIMENTAL DATA. Experiments performed have shown differences in the behavior of iron(II) and iron(III) with nitroso-R salt.

As shown in Table IV, it required less iron(III) than iron(II) to cause a depression in the absorbance of cobalt–nitroso-R salt.

The absorbance of the iron(III)–nitroso-R salt chelate is a function of the concentration at the time of formation as shown in Table V, whereas the absorbance of the iron(II) chelate with the reagent is independent of concentration at the time of formation.

The depression of the absorbance of cobalt–nitroso-R salt by iron(II) may be eliminated by adding hydrochloric acid and sodium acetate alternately after color development; however, the effect of iron(III) cannot be eliminated by this treatment.

The depression of the absorbance of cobalt–nitroso-R salt caused by iron(III) can be eliminated by heating the buffered solution before the addition of the reagent, as shown in Table VI.

The depression caused by iron(II) cannot be eliminated in this way.

The depression of the absorbance of cobalt–nitroso-R salt caused by iron(II) can be eliminated by use of additional reagent. Excess nitroso-R salt enhances the effect of iron(III) as shown in Table VII.

Cobalt added to iron(II)–nitroso-R salt, which is subsequently destroyed by nitric acid, results in subnormal absorbance when there is insufficient reagent. When added to the complex of iron(III), it produces normal transmittance as shown in Table VIII.

Absorption studies as illustrated in Figure 1 show that iron-(II) and iron(III) produce the same compound with nitroso-R salt in buffered solutions, but more nitroso-R salt is required with iron(III) than with iron(II) to obtain the same absorbance.

Iron reacts with nitroso-R salt in two ways when buffered with sodium acetate at pH 5.5: Iron(II) forms a green complex rapidly and iron(III) forms a green complex slowly. It is probable that in the case of iron(III), an oxidation-reduction reaction is occurring in addition to complex formation. To determine the role of the oxidation and reduction reactions in the depression of the absorbance of cobalt–nitroso-R salt by iron, this reaction was studied in the presence of varying quantities of stronger oxidants and reductants which do not complex nitroso-R salt. The results of these studies are shown in Table IX.

Quantities of hydroxylamine varying from zero to 750 mg. were added, and no depression of the absorbance was observed up to 100 mg. in solutions heated to boiling. However, 200 mg. and above produced a considerable depression which varied with time and temperature rather than concentration. Cobalt–nitroso-R salt color will fade if boiled long enough with over 25 mg. of hydroxylamine. To determine whether this fading of the cobalt complex in the presence of such large quantities of hydroxylamine was the result of the ionization of cobalt, additional nitroso-R salt was added to a solution after the color of the complex had been completely destroyed by the hydroxylamine. There was no change in color; however, it was uncertain whether this was the result of the destruction of reagent with excess hydroxylamine or whether the cobalt originally present was no longer available for reaction. The addition of more cobalt produced a color indicating that the cobalt originally present had complexed.

To determine the mechanism of reduction by hydroxylamine, nitroso-R salt was boiled for 5 minutes with hydroxylamine and cooled to room temperature. When cobalt(II) was added to this mixture, the normal color reaction for cobalt occurred. This color faded when the solution was boiled, but was restored by the addition of a weak solution of potassium permanganate. Furthermore, iron(II)–nitroso-R salt was not decolorized by boiling with hydroxylamine proving that hydroxylamine reduces the metal ion rather than the organic molecule.

Table IX shows that less than 1 mg. of ammonium persulfate causes a depression of the absorbance of the cobalt–nitroso-R salt. The ob-

**Table VII. Effect of Excess Nitroso-R Salt upon Depression of Absorbance by Iron**[a]

| Fe$^{+++}$, $\gamma$ | Fe$^{++}$, $\gamma$ | Absorbance × 100 5 Mg. nitroso-R salt | Absorbance × 100 10 Mg. nitroso-R salt |
|---|---|---|---|
| 40 | .. | 65.0 | 58.0 |
| 80 | .. | 58.9 | 58.0 |
| 200 | .. | 58.1 | 55.0 |
| 400 | .. | 58.1 | 54.0 |
| .. | 249 | 63.0 | 63.0 |
| .. | 400 | 53.2 | 63.0 |
| .. | 498 | 58.0 | 63.0 |

[a] Concentrations. 40 γ of cobalt per 25 ml., 0.66 gram of sodium acetate per 25 ml.

**Table VIII. Effect of Order of Addition of 40 γ of Cobalt upon Absorbance of Cobalt–Nitroso-R Salt in Presence of Iron(II) and Iron(III)**[a]

| Fe$^{+++}$, $\gamma$ | Fe$^{++}$, $\gamma$ | Absorbance × 100 Before color development | Absorbance × 100 After color development |
|---|---|---|---|
| 40 | .. | 65.0 | 65.0 |
| 80 | .. | 58.9 | 64.0 |
| 200 | .. | 58.1 | 64.0 |
| 400 | .. | 58.1 | 63.8 |
| .. | 249 | 63.0 | 48.0 |
| .. | 400 | 53.2 | ... |
| .. | 498 | 58.0 | 28.0 |

[a] Concentrations. 40 γ of cobalt per 25 ml., 0.66 gram of sodium acetate per 25 ml.

served effect is greater when the oxidant is present initially than when it is added after color development; however, in both cases a depression of the absorbance occurred. This depression is further enhanced by adding the oxidant to nitroso-R salt before the cobalt is added thereby indicating that oxidizing agents attack free nitroso-R salt.

If iron(II) and iron(III) were reacting solely as oxidizing or reducing agents, their behavior would be similar but less intense than that of hydroxylamine or ammonium persulfate. Table I shows that 400 γ or iron(II) will cause a depression of absorbance while 60 γ of iron (III) will have a similar effect. This amount of iron(II) is considerably less than the minimum effec-
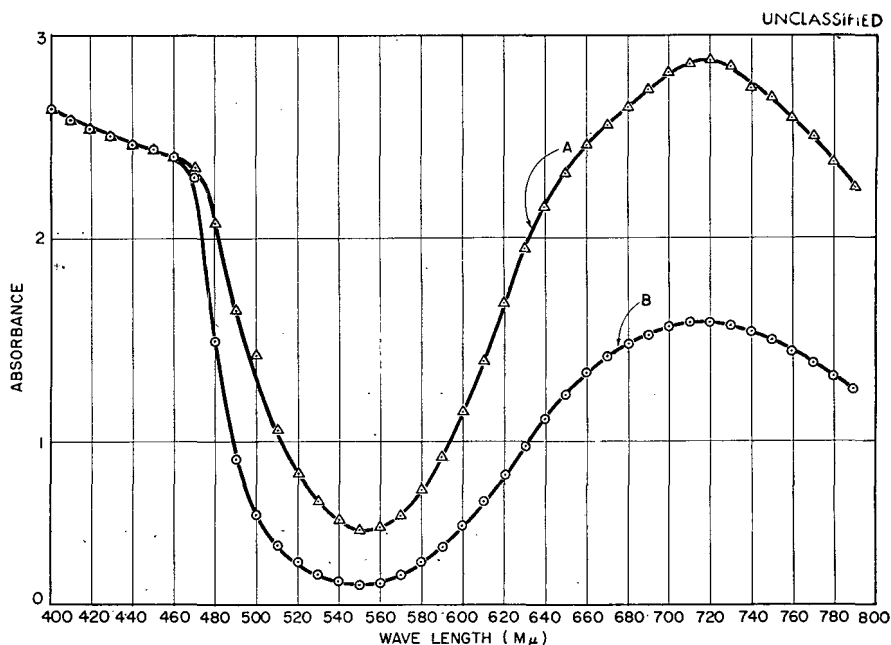
**Figure 1. Absorption spectra of nitroso-R salt complex of iron**

Curve transcribed from data obtained with Cary Model 11 recording spectrophotometer, slit width control 25, using 10.0-cm. cuvettes with distilled water as reference solution
A. 50 γ of iron(II), 2 ml. of sodium acetate, 1 ml. of nitroso-R salt at pH 5.5
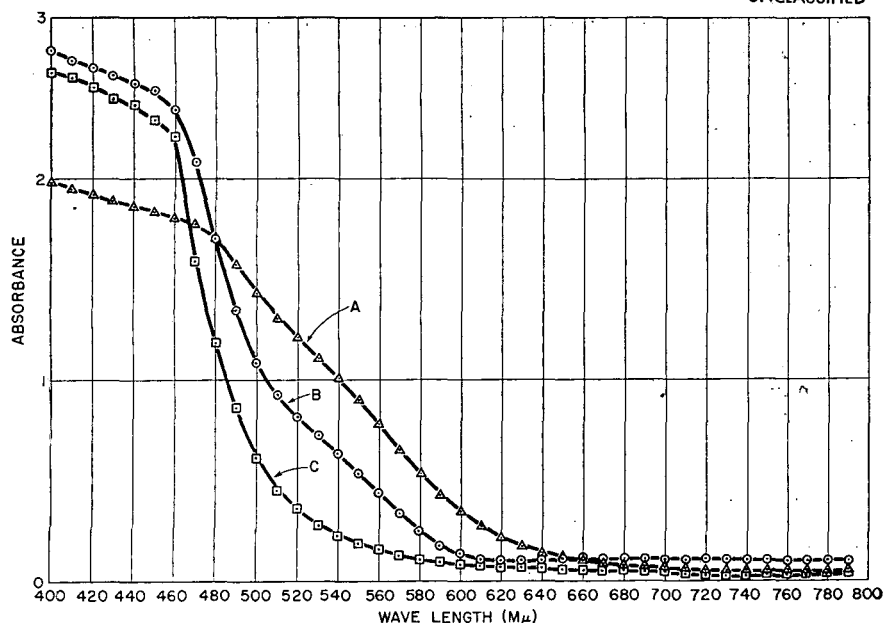B. 50 γ of iron(III), 2 ml. of sodium acetate, 1 ml. of nitroso-R salt at pH 5.5

**Figure 2. Absorption spectra of nitroso-R salt complex of iron and cobalt**

Curve transcribed from data obtained with Cary Model 11 recording spectrophotometer, slit width
control 25, using 10.0-cm. cuvettes with distilled water as reference solution
A. 400 γ of iron(III), 40 γ of cobalt(II), 1 ml. of nitroso-R salt
B. 40 γ of cobalt(II), 2 ml. of sodium acetate, 1 ml. of nitroso-R salt at pH 5.5
C. 400 γ of iron(III), 1 ml. of nitroso-R salt

since a depression in the absorbance of the cobalt compound does occur. Since the interference of iron(III) cannot be convincingly established as an oxidation phenomenon, the formation of a stable mixed complex is at present the only phenomenon which might explain the differences in the behavior between iron(II) and iron(III). Iron(III) forms a reddish brown complex in unbuffered solutions. This complex is destroyed by nitric acid and is converted to a green complex by sodium acetate in the presence of excess nitroso-R salt.

Table XI shows that in buffered solutions where a shortage of nitroso-R salt exists, iron(III) is not completely converted to a green complex signifying that a portion of the reddish brown complex may still exist at pH 5.5. When an unbuffered mixture of iron-(III) and cobalt(II) is treated with nitroso-R salt, a reddish brown complex is formed which is stable to nitric acid. The absorption spectra in Figure 2 show that this compound differs from both cobalt–nitroso-R salt and iron(III)–nitroso-R salt indicating the existence of a stable mixed chelate.

DISCUSSION OF DATA. A stable mixed complex seems to be formed when iron(III) and cobalt(II) are added to nitroso-R salt. Assuming that the cobalt complex contains three chelated molecules of reagent, one might postulate the structure shown in Figure 3 for the mixed complex.
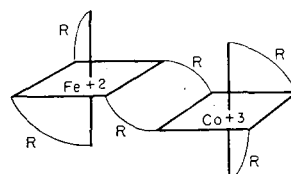
**Table IX. Effect of Oxidants and Reductants upon Absorbance of Cobalt–Nitroso-R Salt[a]**

| Interfering Ions, Mg. | Absorbance × 100 | |
|---|---|---|
| | NH₂OH.HCl | (NH₄)₂S₂O₈ |
| 0 | 61.5 | 63.8 |
| 0.1 | 61.5 | 63.6 |
| 0.2 | 61.0 | |
| 1.0 | 60.5 | 56.2 |
| 10.0 | 61.0 | 36.5 |
| 100.0 | 61.0 | .. |
| 200.0 | 50.0 | .. |

[a] Concentrations. 40 γ of cobalt per 25 ml., 5 mg. of nitroso-R salt per 25 ml.

**Table X. Reaction of Cobalt–Nitroso-R Salt with Oxidized Nitroso-R Salt[a]**

| H₂O₂, Ml. | Absorbance × 100 | |
|---|---|---|
| | A[b] | B[c] |
| 0 | 65.0 | 64.8 |
| 1 | 46.5 | 61.2 |
| 2 | 11.2 | 60.3 |
| 3 | 9.7 | 59.0 |
| 4 | 9.4 | 59.0 |

[a] Concentrations. 40 γ of cobalt per 25 ml., 5 mg. of nitroso-R salt per 25 ml., 1.4% hydrogen peroxide per ml.
[b] 40 γ of cobalt added before addition of 1 ml. of oxidized nitroso-R salt.
[c] 40 γ of cobalt added before addition of oxidized nitroso-R salt plus 1 ml of nitroso-R salt.



**Figure 3. Postulated mixed chelate structure where R represents nitroso-R salt**

**Table XI. Effect of Reagent Shortage upon Nitroso-R Salt Complex of Iron(III)**

| Fe⁺⁺⁺, γ | Absorbance × 100 | |
|---|---|---|
| | 1 Ml. nitroso-R salt | 2 Ml. nitroso-R salt |
| 20 | 13.0 | 12.4 |
| 40 | 26.6 | 25.0 |
| 60 | 32.8 | 36.3 |
| 80 | 35.5 | 49.1 |
| 100 | 35.5 | 61.2 |
| 200 | 45.0 | 108.0 |

tive amount of hydroxylamine. The minimum amount of iron-(III) is lower but in the same order of magnitude as ammonium persulfate. This fact indicates that iron(III) might act as an oxidizing agent but that iron(II) does not act as a reducing agent with respect to nitroso-R salt. If this indication is true, one would expect iron(III) to produce oxidized nitroso-R salt. If this product forms a stable complex with cobalt, a depression in the absorbance at 525 mμ would occur.

Table X shows that most of the cobalt which has been treated with oxidized nitroso-R salt still reacts with the reagent, indicating that it does not form a stable complex with the oxidation product. These data do not preclude the foregoing hypothesis,

This complex is apparently stabilized by internal oxidation of cobalt and is resistant to destruction by strong acids. Because the absorbance of this complex is lower than that of the pure cobalt complex, a depression of absorbance is observed at 525 mμ when iron(III) is present. Complexed iron(III) does not form this compound. The acetate complexes only a small amount of iron(III) at room temperature; however, when added to a hot mixture of iron(III) and cobalt(II), no depression in absorbance is observed. When the brown complex of iron(III) is treated

### Table XII.  Summary of Interfering Cations

| Interfering Ion | Limit of Interference, $\gamma$ | Cause of Interference | Elimination of Interference |
|---|---|---|---|
| Fe$^{++}$ | 400 | Shortage of nitroso-R salt | Additional nitroso-R salt |
| Fe$^{+++}$ | 60 | Possible stable mixed complex | Complexation |
| Ce$^{++++}$ | 100 | Possible stable mixed complex | Complexation |
| Cr$^{+++}$ | 400 | Color of Cr$^{+++}$ | Complexation |
| Cr$^{++++++}$ | 40 | Possible stable mixed complex$^a$ | Complexation |
| Ni$^{++}$ | 2400 | Competition for nitroso-R salt$^a$ | Complexation |
| Sn$^{++}$ | 1000 | Reduction | Bromination of sample |
| VO$^{++}$ | 1000 | Reduction | Bromination or complexation |
| Cu$^{++}$ | 300 | Competition | Complexation |

$^a$ Cause of interference not definitely established.

### Table XIII.  Cobalt in Steel Samples

| Sample | Cobalt Present, % | Average Cobalt Found, % | Standard Deviation, % | Number of Determinations |
|---|---|---|---|---|
| 8G | 10.0 | 10.0 | 0 | 6 |
| 153 | 8.43 | 8.28 | 0.21 | 6 |
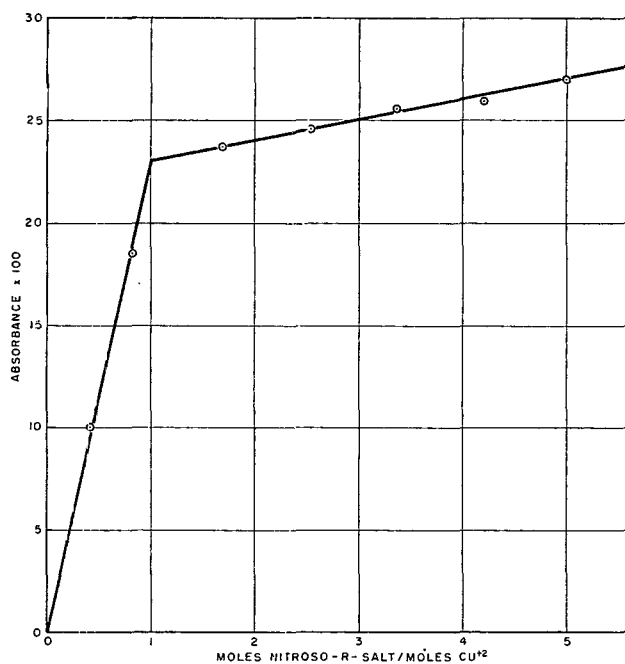


Figure 4.  Photometric titration of copper(II) with reagent in an unbuffered medium



Figure 5.  Photometric titration of copper(II) with reagent in a medium buffered to pH 5.5

with sodium acetate and additional nitroso-R salt, it is converted slowly to iron(II)–nitroso-R salt. After iron(III) has reacted with the reagent or with sodium acetate, cobalt can then be added and its full color development will be attained without interference.

Iron(II) does not form stable mixed complexes with cobalt and is not complexed by acetates and fluorides. The interference of iron(II) is probably based upon the rate of its reaction with nitroso-R salt. Cobalt reacts more rapidly with nitroso-R salt than iron(II) for in the presence of large quantities of iron(II) a relatively small depression in absorbance is observed. If the iron(II) complex is destroyed by hydrochloric acid, nitroso-R salt is released and becomes available to unreacted cobalt(II). Upon raising the pH of the solution, the small amount of unreacted cobalt takes up the released nitroso-R salt and no interfer-

ence is observed after the iron(II) complex is destroyed again. The interference by iron(III), where a stable double complex with cobalt is formed, cannot be eliminated by the above procedure. The interference of iron(II) can be eliminated by the addition of excess nitroso-R salt and much larger quantities of iron(II) than iron(III) can be tolerated without interference, because it forms no stable mixed complex.

A variety of procedures may be used for the analyses of cobalt with nitroso-R salt in the presence of iron depending on the quantity and valence state of the iron present.

**Interference of Cerium(IV).** Cerium(IV) forms a thermally unstable red complex with nitroso-R salt which decomposes upon heating. When cobalt is present, a brownish red mixed complex forms which is stable to heat as well as strong acids. The resulting depression in absorbance can be eliminated by complexing the cerium with sodium acetate or potassium fluoride before the addition of reagent.

**Interference of Chromium(III).** Chromium(III) forms a brownish red complex with nitroso-R salt only after the solution has been heated. It does not interfere with the formation of pure cobalt–nitroso-R salt but its complex is more stable to nitric acid than the complex of iron(II). After its destruction with nitric acid, colored chromium(III) ions are released which cause high results. This interference can be eliminated by complexation with potassium fluoride.

**Interference of Chromium(VI).** Chromium(VI) forms no colored complex with nitroso-R salt. A stable mixed complex of this ion with cobalt may be proposed because of the similarity of its behavior to that of cerium(IV) and iron(III) in the presence of cobalt and nitroso-R salt. The interference of chromium(VI) can be eliminated by complexation with acetate or fluorides.

**Interference of Nickel.** Nickel forms an acid unstable brown complex with nitroso-R salt. When present in fairly large quantities, it causes low results. Its interference is not remedied by addition of excess nitroso-R salt. Fluoride ions precipitate the nickel and eliminate its interference.

**Interference of Copper.** Copper combines with nitroso-R salt in an unbuffered medium to form a soluble brown compound which contains 1 mole of nitroso-R salt for each mole of copper as can be seen in Figure 4. This agrees with the observations made by Dean (2). The chelates of copper in the presence of acetate buffered to a pH of 5.5 appear to exhibit more than one complex as shown in Figure 5.

**Interference of Vanadium(IV).** Vanadyl ion acts as a reducing agent when present in high concentration. Its interference can be eliminated by oxidation with bromine or by the addition of potassium fluoride.

**Interference of Tin(II).** Tin(II) forms a colorless complex with nitroso-R salt and also acts as a reducing agent. Its interference can be eliminated by oxidation with bromine.

Table XII summarizes the study of cations which interfere with the cobalt–nitroso-R salt reaction.

If samples are previously brominated and alkali-fluoride is used as the complexing agent, cobalt may be determined with nitroso-R salt without interferences.

The results obtained for cobalt in two steel samples from the National Bureau of Standards are shown in Table XIII.

The results of this investigation and the methods employed may be applicable to other colorimetric procedures.

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# Conductometric Determination of Phenolic Groups in Mixtures Such as Isolated Lignins

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Of the methods available for the determination of phenolic groups, the conductometric method seems to have special advantages for complex mixtures. A study of the effects of temperature, time, concentration, solvent, and phenolic structure has led to a technique suitable for soluble lignins as well as simple phenols. The reproducibility is good and accurate results can be obtained except when very weak phenolic groups are present. The results, which correspond at least to a minimum value for the content of weakly acidic functional groups, have been compared with the results obtained by other methods on similar lignins.

THE quantitative determination of phenolic groups has been the subject of many investigations (Table I) and in particular on lignin a variety of methods have been employed. These methods frequently have given widely divergent results on the same or similar lignin preparations and it has often not been possible to designate one as more reliable than another. This paper discusses possible sources of error in various phenolic group determinations (with special reference to lignin) and presents in detail a conductometric technique for all alkali-soluble lignins which seems to have more promising features than other methods presently used.

The accuracy of determination of functional group content depends on the specificity and completeness of the reaction used and on the method employed to measure the reaction (*11*). In Table I are listed several reactions which have been used for phenolic determinations and the corresponding method of quantitative measurement. Clearly methods 1 through 8 apply satisfactory analytical methods for measurement and any serious error which may be present in these must originate in the specificity or completeness of the reactions. Diazomethane, for example, is known to methylate carbohydrates in the presence of water and to be relatively unreactive toward some phenols such as conidendrin (*6*). Reactions 3, 4, and 5 are not specific for phenols nor do phenols all consume the same amount of reagent. Dinitrofluorobenzene also has been shown not to distinguish clearly between phenolic and alcoholic groups in all cases (*41*) and the sixth method is at present difficult to evaluate.

The precipitation of alkaline solutions of lignin with barium chloride (method 7) may not result in the complete reaction of all phenolic groups with barium, for relatively small amounts of bivalent ions can cause gel formation and precipitation with polymeric acids (*34*). However, reasonable results have been obtained on kraft lignin (*13*, *22*). The measurement of weakly acidic groups in a solid by the absorption of bivalent cations is difficult to evaluate in the absence of independent confirmatory data. These methods are all sufficiently indirect or tedious experimentally to encourage the search for another more general technique for routine analytical purposes.

The reaction of choice for the analysis of phenolic groups should be their neutralization with alkali in homogeneous phase, because of the ease of interpretation of results. The reaction is specific for acidic functional groups, interference from alcoholic hydroxyl is negligible, and the completeness of the reaction is affected by the pK of the phenol and the experimental conditions in a predictable fashion. (Of course, in lignin preparations containing carboxyl and mercaptan groups, these will also be neutralized.)

**Table I.   Methods for Determination of Phenolic Groups**

| No. | Reaction | Method of Measurement | Typical References |
|---|---|---|---|
| 1 | Diazomethane methylation | Methoxyl content after repeated treatments | (*4, 5, 8*) |
| 2 | Dinitrofluorobenzene | Nitrogen content of product | (*14, 16, 41*) |
| 3 | Periodate oxidation | Periodic acid consumption | (*29, 30, 33*) |
| 4 | Bromination | Titrimetric determination of bromine consumption | (*10, 26*) |
| 5 | Acetylation | Titration of liberated acetic acid | (*37*) |
| 6 | Tosylation and reaction with hydrazine | Weight of toluene sulfinic acid formed | (*15, 17*) |
| 7 | Barium chloride precipitation of alkaline lignin solutions | Titration of supernatant liquor | (*13, 22*) |
| 8 | Absorption of barium hydroxide by solid lignin | Titration of supernatant liquor | (*39*) |
| 9 | Neutralization with alkali | Change in ultraviolet absorption upon ionization | (*1, 2, 3, 20*) |
|  |  | Optical titration | (*18, 19*) |
|  |  | Potentiometric titration in nonaqueous solvents | (*12, 21, 22, 23, 28*) |
|  |  | Conductometric titrations | (*24, 25, 29*) |

In practice, difficulties with determinations of this type have arisen more from certain experimental problems and from quantitative measurement than from the reaction itself.

Perhaps the method of measurement studied most extensively on lignin has been dependent on the difference in the ultraviolet absorption of unionized phenol and of the phenolate ion. By comparing the difference between the absorbances of neutral and alkaline solutions of lignins with the same difference obtained for model phenols, an estimation of the phenolic content of the lignin can be made. An extensive study (2, 3) on several lignin samples and model substances has revealed, however, that none of the many model phenols studied give difference spectra similar enough to those of the lignin samples to allow any truly quantitative interpretation.
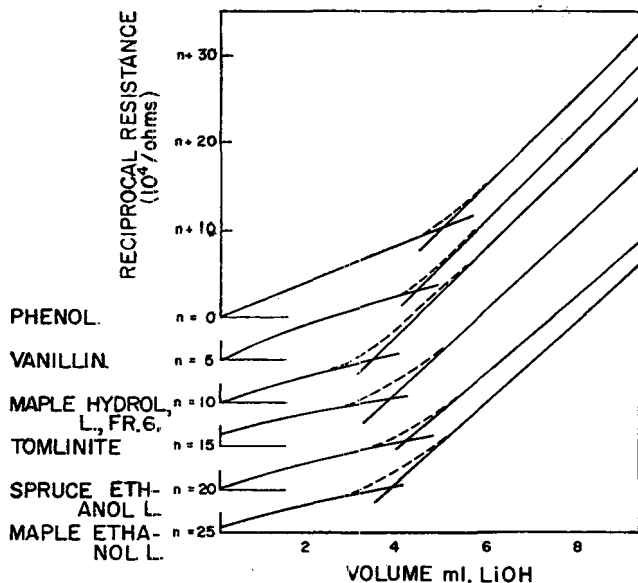


**Figure 1. Typical conductometric titration curves**

Determination of phenols is possible by following the change in absorbance of their solutions during titration with alkali. This technique requires no assumptions regarding the similarity of the substrate and a model—the major weakness of the previous method—and the end point is determined by a linear extrapolation. The high absorbance of lignin solutions, however, makes this method inconvenient and probably inaccurate (19), and, because of the large variations in difference spectra of phenols, probably not valid for most complex mixtures.

The standard potentiometric titration, when applied to phenols using mixed aqueous organic solvents, results in diffuse curves and inexact end points which are only slightly improved by the use of concentrated phenol solution and titrant. The use of anhydrous basic solvents is advantageous for the potentiometric titration of weak acids for their ionization is enhanced and the titration curves are sharpened (28). Swedish authors have published results for ethanol and alkali lignins (12, 22, 23) obtained from titrations in ethylenediamine and pyridine. However, most lignin salts are only slightly soluble in anhydrous media, and precipitation usually occurs during titration and obscures the end point.

Conductometric titrations, which have been recommended by Kolthoff for the determination of phenolic groups (24, 25) and applied to lignin sulfonic acids by Peniston and McCarthy (29), have three important advantages for the titration of the complex mixtures of weak acids found in soluble lignins.

First, typical titration curves (Figure 1) consist of two intersecting approximately straight lines, a salt line of low slope obtained

during the neutralization of the acid functions and a line of high slope obtained after complete neutralization. These lines can be extrapolated to an intersection—the end point—where the experimental points deviate most from the straight lines because of hydrolysis of the phenolate salt. This advantage is not shared by potentiometric titrations.

Secondly, the appearance of hydroxyl ion is measured directly by its own high conductivity in aqueous solutions, rather than by some property of the phenols which may be different for individual components.

Third, under the conditions used the presence of dispersed rather than dissolved lignin in the early stages of the titration does not interfere with the measurement and the solution is always homogeneous before the neutral point is reached.

The only advantage which an alternate method might give would be to permit the use of a more basic solvent system, and the titration of extremely weak phenolic groups. This seems not to be possible with conductometry (32).

### EXPERIMENTAL

**Lignin Preparations.** Native red spruce lignin was obtained from S. E. Gottlieb of the University of Maryland; native aspen lignin from F. E. Brauns of the Institute of Paper Chemistry; Tomlinite, a soda lignin, from George Tomlinson of Howard Smith Paper Co., Cornwall, Ont.; Indulin, a pine kraft lignin, from West Virginia Pulp & Paper Co., New York 17, N. Y.; Meadol, a hardwood soda lignin, from Necmi Sanyer of Mead Co., Chillicothe, Ohio; the spruce ethanol lignin was prepared by Herbert G. Arlt in a stepwise fashion in chloroform-ethanol solution according to directions published previously (36).

The maple ethanol lignin was a petroleum ether–insoluble fraction prepared in the usual fashion (40). The hydrol lignin was prepared according to the directions of Brewer, Cooke, and Hibbert (7) and fractionated by the addition of petroleum ether to a dioxane solution to give nine fractions (number 1 of lowest solubility and highest viscosity). The unfractionated material was partitioned between ethanolic alkali and chloroform (35) to give an acidic and neutral fraction.
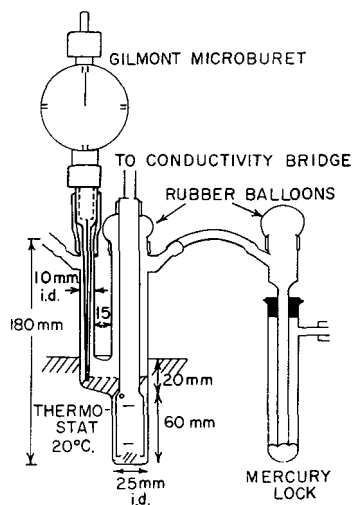


**Figure 2. Conductivity titration apparatus**

**Apparatus and Reagents.** A conductivity bridge, Model RC-1, made by Industrial Instruments, Cedar Grove, N. J., and a glass-mantled dipping-type conductivity cell (cell constant 1.068) with platinized electrodes were used. The borosilicate glass titration cell is shown in Figure 2. In small scale titrations a microconductivity cell was used (cell constant about 2, American Instrument Co., Silver Spring, Md.). The titration cell, constructed of a test tube, was similar in shape to the larger one, but had a capacity of only 7.5 ml. The addition of the titrant was carried out in both cases using a Gilmont microburet (Emil Greiner Co., New York, N. Y.) with a total volume of 1 ml.

The titrant was 2.5$N$ lithium hydroxide solution, prepared by dissolving 26.20 grams of lithium hydroxide monohydrate in distilled water to a volume of 250 ml. It was standardized against potassium acid phthalate using the same buret as in the titrations and stored in a tightly stoppered polyethylene bottle. The organic solvents used were 96% ethanol and c.p. grade acetone.

**Procedure.** A lignin sample corresponding to a final concentration of 0.04 to 0.06 phenolic equivalent per liter is weighed and placed on the bottom of the titration cell. Five milliliters of acetone is added from a pipet, and the sample is allowed to dissolve. Then, 10 ml. of 96% ethanol is added and finally 15 ml. of distilled water, dropwise and mixing cautiously. Some finely divided precipitate is usually formed during the last addition, but it always is completely dissolved before the equivalent point is reached. It is important to ensure that no sticky residue remains undissolved. In the case of kraft lignin, it is most convenient to add the ethanol first.

The titration cell is placed in an automatically controlled constant temperature bath adjusted to +20° C. and is fitted with the buret, the conductivity cell, and nitrogen inlet and outlet tubes (Figure 2). A thin rubber tubing, as from a balloon, connects the conductivity cell to the titration cell and is held tightly in position by two elastic bands. Air is removed from the apparatus by passing about 500 ml. of nitrogen gas through the system (conveniently measured by water displacement) and by then closing the mercury lock. Mixing is obtained during the titration by an up-and-down movement of the conductivity cell, but care must be exercised to avoid entrapping gas bubbles in the cell. The titrant is added in 0.1-ml. portions and after mixing (by 8 to 10 up-and-down movements of the conductivity cell) the electrical resistance is measured and recorded. After every titration it is advisable to discard 0.1 ml. of the residual titrant in the buret to avoid contamination. From the plot of reciprocal resistance values against the alkali addition the equivalent volume can be found in the usual way. Since the added volume is less than 4% of the total, volume corrections are unnecessary.

### DISCUSSION

In developing this described technique, the factors influencing the accuracy and precision of the method were studied extensively and the conclusions reached are described below.

**Effect of Solvent.** Although various polar organic solvents dissolve isolated lignins readily, they cannot be used alone for this determination. A substantial proportion of water must be added for the following reasons.

First, the sharpness of the angle between the two lines obtained in the conductivity plot is greatest in pure water and becomes less as organic solvents are added, because these substances suppress the conductivity of the hydroxyl ion. Figure 3 shows the lines obtained when various organic solvent–water mixtures are titrated with potassium hydroxide. Of these, lower alcohols and acetone cause the least suppression of conductivity and, therefore, allow most precision.

Secondly, the salt and alkali lines should be as straight as possible in order to allow easy extrapolation to the break point. The amount of deviation from linearity of these lines is defined by the value of $k$ in the Kohlrausch equation

$$\Lambda = \Lambda_0 - k\sqrt{C}$$

One factor which increases the constant $k$ is a medium of low dielectric constant and this should be avoided.

**Effect of Base Type.** The conductivity of lithium ion is low and in confirming previous work (31), lithium hydroxide was found to give sharper breaks than sodium, potassium, or trimethylbenzylammonium hydroxide.

**Effect of Titration Time and Temperature.** In lignin titrations, the resistance readings were not found to be constant after passing the equivalence point but slowly increased with time if the addition of titrant was interrupted. At 20° C., the change was slow enough, so that the error was negligible when the titration was carried through without interruption and within reasonable time (10 minutes). However, when the titration temperature was +30° C., errors of about 10 to 15% resulted leading to too high values for the phenolic content. This was demonstrated in the following way: For a sample of maple ethanol lignin an equiva-

lent weight of 370 was obtained in a normal titration at 30° C. When an excess of titrant was added in one portion at the same temperature and the immediately measured conductivity value used for constructing the alkali line (parallel to that obtained by the usual titration), a considerably higher value 431 was obtained. Normal titrations at 20° and 0° C. gave equivalent weights 428 and 432, respectively. If at 20° C. an excess of alkali was added in one portion of the alkali line constructed as before, the equivalent weight was 436, which differs from the normal titration value at the same temperature by less than 2%.
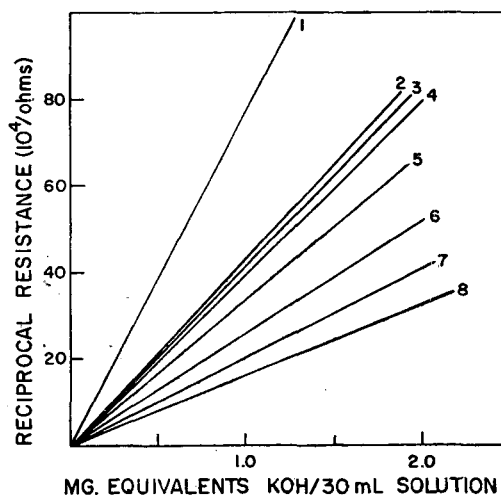


**Figure 3.** **Alkali lines in different media**

1. **Pure water**
2. **Acetone solution, 33%**
3. **Ethanol solution, 33%**
4. **Methanol solution, 33%**
5. **Diacetone alcohol solution, 33%**
6. **Dioxane solution, 33%**
7. **Acetone solution, 66%**
8. **Ethyl Cellosolve solution, 33%**

This conductivity drift in alkaline lignin solutions seems to be general, but is practically nonexistent in solutions of pure phenols. Since the drift occurs with hydrogenated lignin, it cannot be the result of carbonyl enolization; and since it occurs with alkali lignins, new phenolic groups cannot be formed during the titrations. The most probable explanation is, therefore, a purely physical change in the system.

**Effect of Phenol Concentration.** The conductometric titration of weak acids is known to require higher acid concentrations than are necessary in the titration of strong acids (25). The apparent pK value of lignin phenol groups has been estimated to be about 10 by Goldschmid (20). Therefore, the minimum lignin concentration had to be established at which reliable results could be expected. This was done by performing several titrations at different concentrations with known phenols and lignin samples using the conditions mentioned. The results (Figure 4) show that all phenols with pK values 10 or less can be accurately titrated, if the concentration is 0.04 equivalent per liter or more.

The curves for lignin samples are similar and reach an essentially constant value at about the same concentration. The solubility characteristics of lignin samples did not permit extending the study to higher concentrations than 0.07 equivalent per liter. The use of low concentrations results in a considerable error. For maple hydrol lignin, as an example, this error would be of the magnitude of 20% at a concentration of 0.01 equivalent per liter.

**Effect of Phenol Structure.** Under the conditions specified, monohydric phenols and phenol carboxylic acids generally give reliable results (24). Phenol sulfonic acid also is reported to
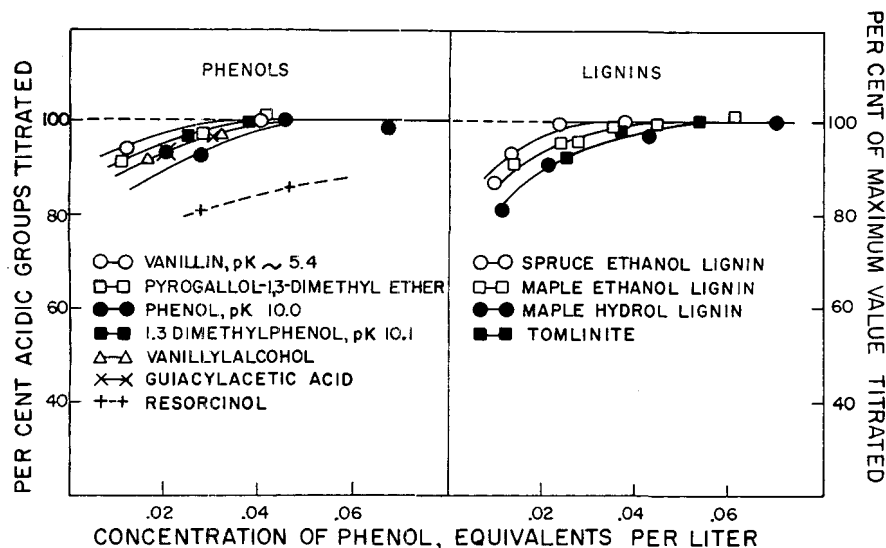
Figure 4. Effect of concentration on conductometric equivalent point

## Table II. Titrations of Low Molecular Weight Phenols

| Phenol | Vol. of Soln., Ml. | Concn. of Phenolic Equiv./L., $\times 10^2$ | Equivalent Weight | | Error, % |
|---|---|---|---|---|---|
| | | | Theor. | Found | |
| Vanillin | 30 | 4.0 | 152 | 152 | 0 |
| Acetovanillone | 30 | 3.5 | 166 | 166 | 0 |
| Vanillyl alcohol | 30 | 4.5 | 154 | 154 | 0 |
| Pyrogallol-1,3-dimethylether | 30 | 4.1 | 154 | 152 | 1.3 |
| Dehydrodiisoeugenol | 7.5 | 3.8 | 326 | 322 | 1.2 |
| Phenol | 30 | 4.6 | 94 | 94 | 0 |
| 3,5-Dimethylphenol | 30 | 3.8 | 122 | 123 | 0.8 |
| Resorcinol | 30 | 4.6 | 55 | 65 | 15 |

titrate as a dibasic acid. Salicylic acid, catechol (24), and other chelated (1, 38), and some sterically hindered (1, 9) phenols may exhibit abnormally low acidities and in part escape titration. Di- and trihydric phenols do not always titrate completely (24). Errors arising from the low acidities of phenols such as these will lead to high neutralization equivalents or low phenolic (and/or acid) contents. There is no obvious source for an error in the opposite direction.

**Results.** Results on some known phenols are recorded in

## Table III. Titrations of Different Lignin Preparations

| Sample | | Equivalent Weight |
|---|---|---|
| Tech. alkali lignins | | |
| 1. | Indulin | 252 |
| 2. | Tomlinite | 277 |
| 3. | Meadol | 244 |
| Ethanol Lignins | | |
| 4. | Maple | 422 |
| 5. | Acidic fraction of 4 | 329 |
| 6. | Neutral fraction of 4 | 597 |
| 7. | Spruce, ether-insoluble | 704 |
| 8. | Spruce, petroleum ether-insoluble (average value of four samples) | 613 |
| Native lignins | | |
| 9. | Aspen | 455 |
| 10. | Red spruce | 407 |
| Hydrol Lignins | | |
| 11. | Maple original ether-insoluble | 744 |
| 12. | Acidic fraction of 11 | 520 |
| 13. | Neutral fraction of 11 | 1472 |
| 14. | Fraction$^a$ D from 11 by precipitation | 968 |
| 15. | Fraction E by precipitation | 831 |
| 16. | Fraction F by precipitation | 759 |
| 17. | Fraction G by precipitation | 605 |

$^a$ Yields of Fractions D, E, F, and G obtained by fractional precipitation of ether insoluble maple hydrol lignin (11) were 12.6, 15.6, 17.4, and 8.5%. Reduced viscosities determined in an Ostwald viscometer at 25.0° at concentration of 3 grams per 100 ml. of dioxane were, respectively, 782, 706, 585, and 481 $\times 10^{-5}$.

Table II. Table III gives the titrimetric equivalent weights of the lignin samples investigated.

The values for technical alkali and spruce ethanol lignins compare well with the earlier potentiometric values for similar samples by Hägglund (22) and by Hägglund and Richtzenhain (23), if differences in preparation are taken into account. The equivalent weight 407 for red spruce native lignin is also in good accordance with the value 420 calculated from the diazomethane methylation data of Brauns for spruce native lignin (4). For aspen native lignin, however, the conductometric and diazomethane methylation values are 455 and 633 (8), respectively. Experience with the diazomethane methylation of hardwood lignins suggests that this discrepancy most probably is due to an incomplete reaction with diazomethane.

The fractions of maple hydrol lignin show a decrease in equivalent weight with increasing solubility and decreasing viscosity of the fractions. This indicates that high molecular weight hydrol lignin fractions generally contain fewer phenolic groups than low molecular weight ones. The values for the alkali-insoluble parts of maple hydrol and maple ethanol lignins show that in organosolv lignins molecular species with widely varying phenolic contents are present.

**Comparison to Spectrophotometric Methods.** Goldschmid has measured the difference in absorbance between alkaline and neutral lignin solutions and assuming that lignin phenols show the same difference in extinction coefficient upon ionization as certain model phenols has calculated the phenolic content of the lignin (20). He has used for this purpose a region of the spectrum
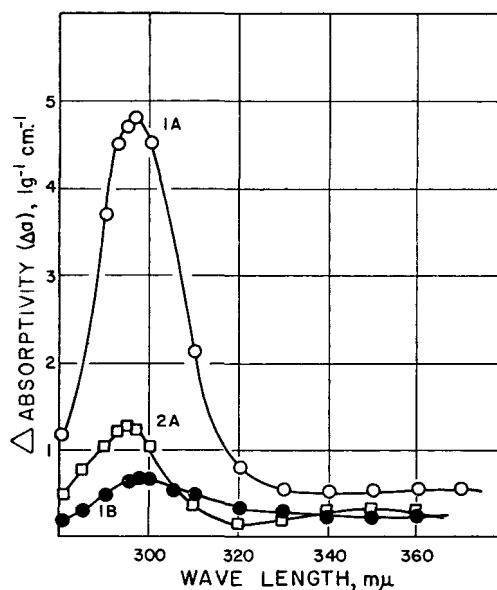


Figure 5. Difference spectra of maple hydrol lignin

1A. Alkali-soluble fraction, pH 6 buffer and 0.1N NaOH
1B. Alkali-soluble fraction, pH 12.3 buffer and 0.5N NaOH
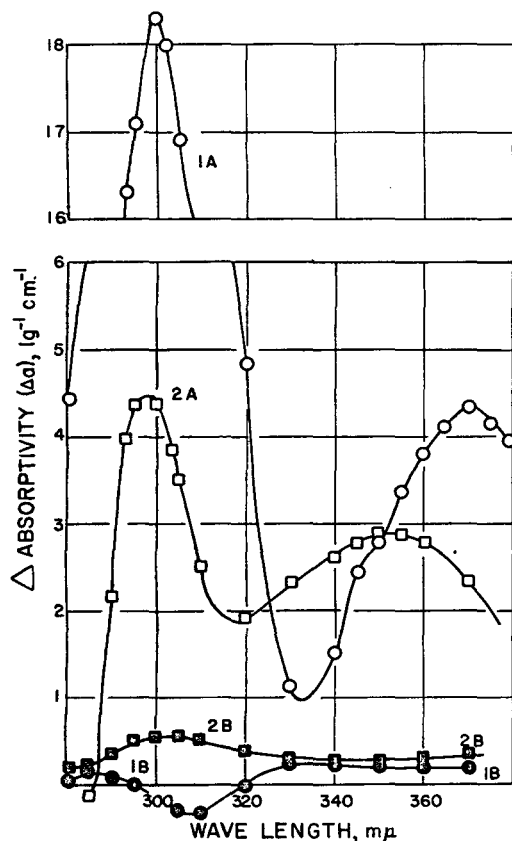2A. Neutral fraction, pH 6 and 12 buffers

Figure 6.  Difference spectra of native
lignins

1*A*.  Aspen, pH 6 and 12 buffers
1*B*.  Aspen, pH 12.3 and 0.5*N* NaOH
2*A*.  Red spruce, pH 6 and 12 buffers
2*B*.  Red spruce, pH 12.3 and 0.5*N* NaOH

Therefore, the use of Goldschmid's method does not seem possible for the quantitative determination of phenolic groups in hardwood lignins.

**Estimate of Accuracy of Method on Various Lignins.** The pH range of the linear portion of the alkali lines in conductometric titrations generally is 12.3 to 12.7. If the dissociation of phenols in this range is incomplete, a difference spectrum run between a lignin solution with pH 12.3 and another solution with high alkali concentration—e.g., 0.5*N*—shows the characteristic maxima of phenol groups. The maxima, as the previous paragraph and the work of Aulin-Erdtman shows (*3*), are only of qualitative
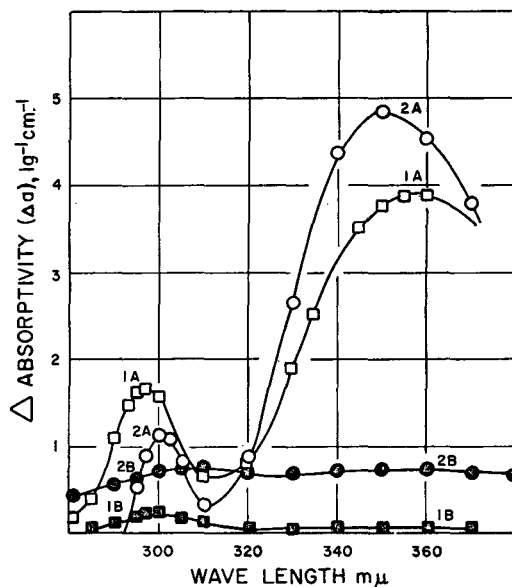


Figure 7.  Difference spectra of ethanol
lignins

1*A*.  Maple, pH 6 and 12 buffers
1*B*.  Maple, pH 12.3 and 0.5*N* NaOH
2*A*.  Spruce, pH 6 and 12 buffers
2*B*.  Spruce, pH 12.3 and 0.5*N* NaOH

(ca. 297) in which unconjugated phenols show a peak and accordingly the method should be most accurate for lignins containing only unconjugated phenols.

Comparison of Goldschmid's method with the conductometric data was made by using a hydrogenated maple lignin (hydrol) which has no peak in the region characteristic of conjugated phenols (ca. 350 m$\mu$; Figure 5. Compare Figures 6, 7, and 8). If the values of the difference in absorptivity at 297 m$\mu$—the wave length showing a maximum in the difference curve—for several hydrol lignin fractions are plotted against the conductometrically determined phenolic content, a fairly good straight-line relationship is found (Figure 9). The average difference in molar absorptivity of phenolic units ($\Delta\epsilon$) calculated from these data, however, is 1840, which is far lower than the value for any known model substance (*2*). In this case, Goldschmid's method gives values for phenolic content lower than authors' method by about 50%. Any error in this method caused by incomplete titration will increase the disagreement.

The difference spectrum of native aspen lignin (Figure 6) shows only a low peak in the region characteristic of conjugated phenols and an exceptionally high peak at 297 m$\mu$. The calculated $\Delta\epsilon$ value 8330 is in this case higher than that of any known model substance. Goldschmid's method, therefore, gives a phenolic content 100% higher than this method, though the error may be somewhat less if this titration is incomplete. (Actually the per cent neutralization of the aspen lignin must be higher in the conductometric titration that in the spectrophotometric determination, since a higher pH is reached.)
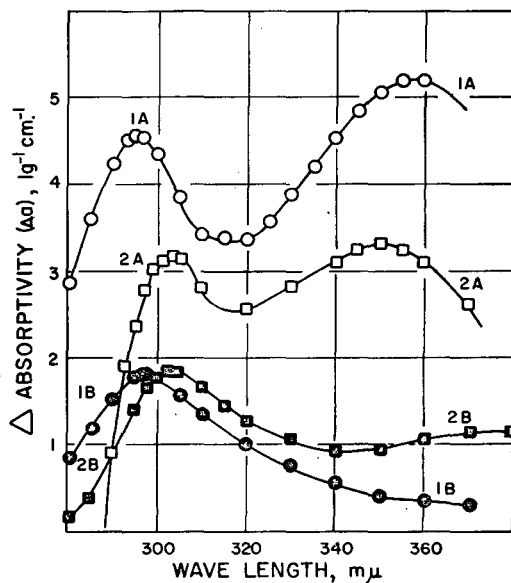


Figure 8.  Difference spectra of alkali lignins

1*A*.  Tomlinite, pH 6 and 12 buffers
1*B*.  Tomlinite, pH 12.3 and 0.5*N* NaOH
2*A*.  Indulin, pH 6 and 12 buffers
2*B*.  Indulin, pH 12.3 and 0.5*N* NaOH

significance. Such comparisons have been made in the following way.

A sample of 100 to 200 mg. of lignin was dissolved in 0.5N sodium hydroxide and made up to 100 ml. with additional 0.5N alkali. Two milliliters of the solution was diluted to 50 ml. with 20% ethanol in water to give approximately pH 12.3. Another 2-ml. sample was diluted to the same volume with 0.5N sodium hydroxide in 20% ethanol. A difference spectrum was obtained using the first as the calibration solution. (When the two alkaline solutions were calibrated separately against an acidified solution, an identical spectrum was obtained by subtraction.)
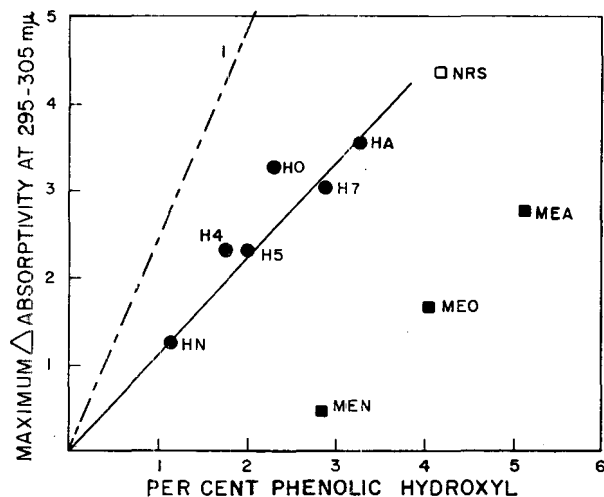


**Figure 9. Maximum Δ absorptivity as function of conductometric phenolic hydroxyl content**

At 295 to 305 mμ, between pH 6 and 12
NRS.   Native red spruce lignin
H.     Maple hydrol lignin
ME.    Maple ethanol lignin
O.     Original sample
A.     Acid fraction
N.     Neutral fraction
I.     Goldschmid's hypothetical line

The spectra obtained are shown in Figures 5, 6, 7, and 8. Three aspects of these spectra are:

1. Essentially no peaks are in the region characteristic of conjugated phenols, which seem to be completely ionized at pH 12.3 and are completely titrated by the authors' method.

2. Small peaks are present in some cases around 300 mμ which probably correspond to very weak unconjugated phenols not completely ionized at pH 12.3 and therefore not completely determined by this method.

3. The absorbance of the lignin solutions is usually enhanced by strong alkali across the entire region of the spectrum shown, over that of the same sample at pH 12.3. As a result regions of the spectra not characterized by peaks—e.g., 340 to 380 mμ—are in most cases above the zero line. This general increase in absorbance is probably not due to further phenolic ionization.

Except for the commercial alkali lignins, the heights—e.g., absorptivity at ca. 300 mμ minus absorptivity at ca. 340 mμ—of the peaks mentioned above which appear in the difference spectra between pH 12.3 and 0.5N alkali are not more and are usually less than 15% of the corresponding peaks shown in Figures 5, 6, 7, and 8. Considering also that the phenolic groups absorbing at 360 mμ have been completely titrated under the conditions used in these cases, the error in total phenolic content should be very small and the conductometric method should give reason-

ably reliable data. In the case of the commercial alkali lignins (Figure 8), the conductometric method must be considered as giving only a maximum value for the equivalent weight.

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# Spectrophotometric Determination of Esters and Anhydrides by Hydroxamic Acid Reaction
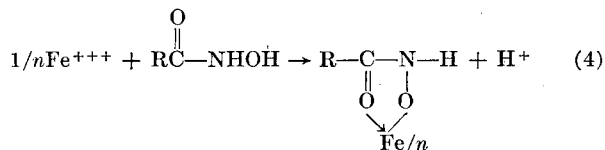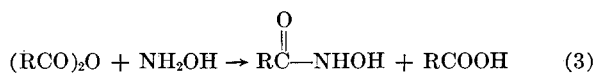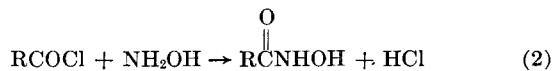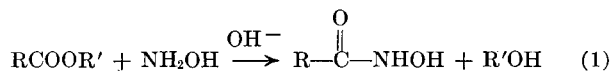
## R. F. GODDU, N. F. LeBLANC, and C. M. WRIGHT

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**The formation of hydroxamic acids from esters or anhydrides by reaction with hydroxylamine in alkaline solution may be used as the basis for a spectrophotometric determination. The hydroxamic acid forms a highly colored chelate complex with ferric ion. This paper reports a study of the reaction variables and an extension of the method to esters of aromatic acids and to the use of a variety of solvents. The absorption maxima and molar absorptivities of some 20 different esters are reported. Conditions have been established under which it is possible to determine anhydrides or lactones by their selective reaction in the presence of esters. For esters or anhydrides alone, the precision and accuracy is within $\pm 2\%$. When the two are present in admixture the results are less accurate. The optimum concentration range of the procedure is $10^{-3}$ to $10^{-2}M$. Acids, most amides, and nitriles do not interfere with this procedure. High concentrations of carbonyls, transition elements, and ions or compounds which complex ferric ion may affect the intensity of the color.**

A N EXAMINATION of the literature shows a scarcity of chemical methods for the determination of small amounts of esters, anhydrides, and other hydrolyzable derivatives of carboxylic acids. Most of the available methods with the exception of the one used as a basis for this paper utilize some variation of a saponification number (7). The work described was undertaken to develop a rapid, sensitive, and relatively selective method for determining hydrolyzable derivatives of carboxylic acids.

An organic ester, acid chloride, or anhydride may form a hydroxamic acid by the reactions shown in Equations 1 to 3. Most hydroxamic acids combine with ferric ion to form characteristic red to purple chelate complexes (Equation 4) which can be measured spectrophotometrically. Work is currently under way to define more clearly the value of $n$ in the ferric-hydroxamate complex of Equation 4 (3)

$$RCOOR' + NH_2OH \xrightarrow{OH^-} R{-}\overset{O}{\overset{\|}{C}}{-}NHOH + R'OH \quad (1)$$

$$RCOCl + NH_2OH \rightarrow R\overset{O}{\overset{\|}{C}}NHOH + HCl \quad (2)$$

$$(RCO)_2O + NH_2OH \rightarrow R\overset{O}{\overset{\|}{C}}{-}NHOH + RCOOH \quad (3)$$

$$1/n Fe^{+++} + R\overset{O}{\overset{\|}{C}}{-}NHOH \rightarrow \underset{\underset{Fe/n}{\overset{\diagdown \diagup}{O \quad O}}}{R{-}\overset{\|}{C}{-}\overset{|}{N}{-}H} + H^+ \quad (4)$$

Hydroxamic acids were first reported in 1869 by Lossen (12), who observed that they could be rearranged to isocyanates (the well-known Lossen rearrangement). However, it was not until 1934 that the analytical possibilities of the hydroxamic acids were widely exploited, when Feigl and coworkers (6) reported a spot test for esters and anhydrides based on the color reaction described. Since then, the reaction has been used to detect and determine many types of esters (1, 4, 8, 9, 11, 17), amides (2, 14, 16), anhydrides (5, 6), and nitriles (16). However, very little of this previous work has been concerned with a systematic study of the reaction variables in order to ascertain optimum conditions for analytical determinations.

## APPARATUS

A Beckman Model B spectrophotometer with 1-cm. cells was used for this work. For routine work a colorimeter with a 525-m$\mu$ filter is satisfactory.

Erlenmeyer flasks with $\mp$ 19 $\times$ 22 glass joints, 25-ml. capacity. Small condensers with $\mp$ 19 $\times$ 22 glass joints.

## REAGENTS

Hydroxylamine hydrochloride, 12.5%, in methanol.
Sodium hydroxide, 12.5%, reagent grade, in methanol.
Both hydroxylamine hydrochloride and sodium hydroxide are prepared by refluxing 12.5 grams of the solid material with 100 ml. of methanol for a few minutes. The sodium hydroxide solutions usually are cloudy because of precipitated sodium carbonate. The hydroxylamine hydrochloride solution is about 1.8$M$ and the sodium hydroxide is 3.1$M$.

**Ferric Perchlorate.** STOCK SOLUTION. Dissolve 5.0 grams of ferric perchlorate (nonyellow, G. F. Smith Chemical Co.) in 10 ml. of 70% perchloric acid and 10 ml. of water. Dilute to 100 ml. with anhydrous 2B alcohol, cooling under a tap as the alcohol is added.

Alternatively, weigh out 0.8 gram of iron wire into a 50-ml. beaker. Add 10 ml. of 70% perchloric acid and heat on a hot plate at low heat until the iron dissolves. Be careful, because the iron dissolves very rapidly when the acid is hot. Cool the beaker and transfer the contents to a 100-ml. volumetric flask with 10 ml. of water and dilute to volume with anhydrous 2B alcohol, cooling under a tap as the alcohol is added.

REAGENT SOLUTION. Prepare the reagent solution by adding 40 ml. of stock solution to a 1-liter volumetric flask. Add 12 ml. of 71% perchloric acid and dilute to volume with anhydrous 2B alcohol. The dilution should be carried out by adding the alcohol in 50- to 100-ml. increments and cooling between each addition until the perchloric acid has been diluted to about 10% of its original concentration. The ferric ion concentration of this solution is 5.7m$M$ and the acid concentration is 0.16$M$.

All esters were used without extensive purification and were of a purity roughly equivalent to Eastman grade of Distillation Products Industries.

## RECOMMENDED PROCEDURES

**Procedure for Determination of Esters.** The alkaline hydroxylamine reagent is prepared by mixing equal volumes of 12.5% hydroxylamine hydrochloride and 12.5% methanolic sodium hydroxide and filtering off the precipitated sodium chloride on Whatman No. 40 paper. The clear filtered reagent solution is usable for 4 hours.

The sample should be dissolved in anhydrous 2B ethanol or one of the other solvents mentioned below. The concentration of substance to be determined should be between 0.01 and 0.001$M$.

Five milliliters of the sample solution is pipetted into a 25-ml. flask with a ground-glass joint. (If a more concentrated solution is to be analyzed, or if a calibration curve is to be drawn up, a smaller volume of sample should be used and enough solvent added to make the total volume of 5 ml. in the flask—e.g., 2 ml. of sample and 3 ml. of solvent.) Three milliliters of the filtered alkaline reagent solution is added to each sample flask, and to a blank which contains 5 ml. of solvent. A boiling chip is added to each flask, then the flasks are placed on a hot plate on low heat, and reflux condensers are attached. The samples are refluxed for 5 minutes. Then the flasks are removed from the hot plate (the condensers are not washed down), cooled to room temperature, and the contents washed into a 50-ml. volumetric flask with the

ferric perchlorate reagent. The samples are made up to volume with the reagent. The flasks are shaken to ensure complete solution of the initially precipitated ferric hydroxide. After several minutes the absorbance of the samples should be read against the reagent blank on a suitable spectrophotometer or colorimeter. The wave length of maximum absorbance varies according to the type of ester. A calibration curve should be drawn up using the same ester as that to be determined or, less preferably, an ester of the same acid as the acid portion of the ester to be determined. The same solvent should be used in all cases.
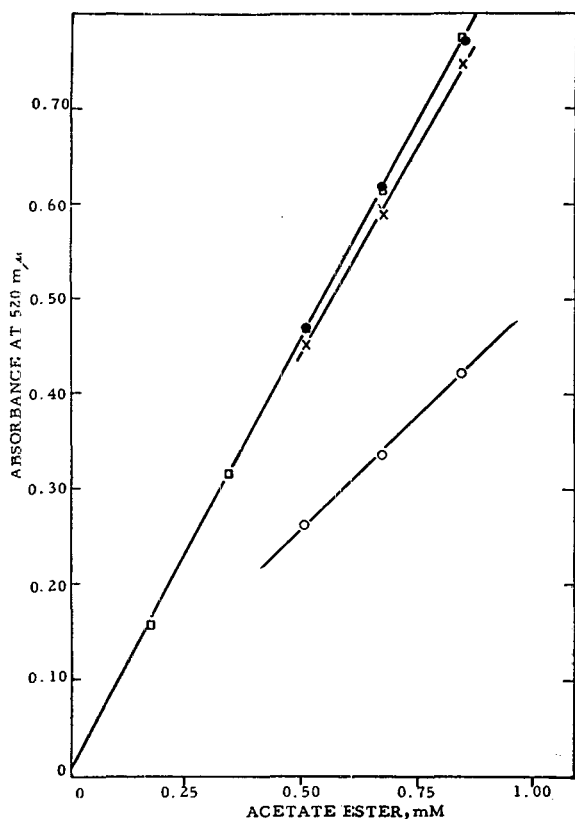


**Figure 1.    Effect of time of reaction between acetate ester and NH₂OH at 25° C.**

Ferric ion concentration, 2.1mM
Hydrogen ion concentration, 0.3M
Reaction times
    O   3 minutes
    X   10 minutes
    ●   15 minutes
    □   30 minutes

If a mixture of anhydride and ester is to be analyzed for both constituents, a calibration curve for the anhydride must also be run under the conditions for the ester determination. The concentration of anhydride is determined under neutral hydrolysis conditions. Then the absorbance due to that concentration of anhydride under basic hydrolysis conditions is subtracted from the observed absorbance prior to calculating the ester content.

**Procedure for Anhydrides and Lactones in Presence of Esters.** The hydroxylamine reagent is prepared by neutralizing a portion of the methanolic hydroxylamine hydrochloride to a phenolphthalein end point by the addition of the 12.5% methanolic sodium hydroxide. The precipitated sodium chloride is filtered on Whatman No. 40 paper. The clear filtered reagent is usable for at least 4 hours.

The sample, if anhydride, should be dissolved at a concentration of 0.01 to 0.001M in benzene which has been suitably dried— for example, dried over anhydrous calcium sulfate for 24 hours. Lactones and esters may be dissolved in any of the ethers, alcohols, or hydrocarbons referred to below.

The procedure for carrying out the analysis is exactly the same as that used for the ester determination, except that a 10-minute reflux time should be used. Calibration curves should be made

with known concentrations of anhydride or lactone by plotting the observed absorbance against concentration. The calibration curves are not always straight lines under these neutral conditions.

**Investigation of Hydroxylaminolysis Conditions.** The determination of esters involves the reaction of an ester with hydroxylamine in alkaline solution to form a hydroxamic acid. An acid ferric perchlorate solution is then added to form the colored chelate complex.

Previous investigators had found that many esters would react at room temperature, so the first variable considered was reaction time at 25° C., rather arbitrarily using 12.5% solutions of hydroxylamine hydrochloride and sodium hydroxide in methanol, concentrations used by Thompson (17). It was found that for acetate esters dissolved in anhydrous 2B alcohol maximum color development was obtained in a minimum of 15 minutes (Figure 1).

However, with other esters, such as fatty acid esters and esters of aromatic acids, maximum color development was not obtained after 30-minute reaction time in alkaline solution at 25° C. Accordingly, the effect of temperature and time were studied over a wider range, as shown in Table I. At elevated temperatures the reaction to form the hydroxamic acid proceeds more swiftly, but prolonged exposure to high temperature may cause decomposition of the hydroxamic acid. For general use, a 5-minute reflux (temperature approximately 72° C.) has been found most satisfactory and applicable to all cases in which it is possible to form a colored ferric hydroxamate complex. Room temperature reactions may be used for limited number of esters.
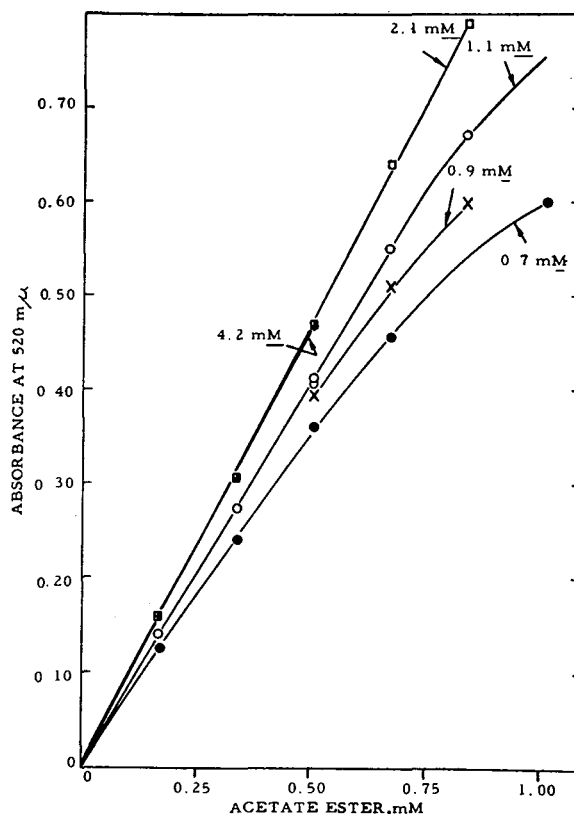


**Figure 2.    Effect of ferric ion concentration on sensitivity of acetate ester determination**

Reaction time, 30 minutes
Hydrogen ion concentration, 0.3M
Ferric ion concentrations
    ●   0.7 mM
    X   0.9 mM
    O   1.1 mM
    □   2.1 mM
    +   4.2 mM

## Table I. Effect of Time and Temperature on Hydroxamic Acid Formation

| Time, Min. | Methyl Oleate $A_{520}$ | | | Methyl p-Toluate $A_{550}$ | | |
|---|---|---|---|---|---|---|
| | At 26° C. | At 55° C. | At reflux | At 26° C. | At 55° C. | At reflux |
| 5 | 0.670 | 0.858 | 0.963 | 0.290 | 0.630 | 1.058 |
| 10 | 0.808 | 0.880 | | 0.480 | | |
| 15 | 0.860 | 0.753 | | 0.625 | | |
| 30 | 0.940 | | | 0.845 | | |

Color development conditions were hydrogen ion concentration $0.3M$ and ferric ion concentration $2.4mM$. $A\lambda$ = absorbance at wave length $\lambda$.

**Investigation of Color Development Conditions.** There are two independent variables in the color development conditions—the concentration of ferric ion used to complex the hydroxamic acid and the concentration of hydrogen ion present in the solution.

An investigation of the effect of the concentration of ferric ion necessary for maximum color development (Figure 2) showed that maximum color is obtained with ferric ion concentrations equal to or greater than $2mM$. In the final procedure the ferric concentration is $4.8mM$. Since the usual concentrations of esters in the color development solution are from 0.1 to $1.0mM$, there is at least a 4.8-fold molar excess of ferric iron present at all times.

The second variable in the color development was the amount of excess acid desirable over that necessary to neutralize the sodium hydroxide required for the saponification. The curves in Figure 3 indicate that too high an acidity will hinder color development, but at acidities less than $0.6M$ satisfactory colors are obtained. A closer investigation of the effect of acidity as shown in Table II indicates that at low acidities the color from a given sample is marginally more intense, and that the color is much more stable. As a result, a procedure was adopted in which the acid concentration after neutralization of the sodium hydroxide is $0.1M$. The colors thus formed are stable for several hours. Previous ferric hydroxamic acid procedures have been plagued with color instability unless hydrogen peroxide was added to eliminate the excess hydroxylamine, as suggested by Hill (9).

## Table II. Effect of Hydrogen Ion Concentration on Color Intensity and Stability of Ferric–Acethydroxamic Acid Complex

(4.8 m$M$ ferric ion; 0.76 m$M$ butyl acetate)

| Molarity of Perchloric Acid | Absorbance at 530 m$\mu$ | Fading Rate, % per Hour |
|---|---|---|
| 0.036 | 0.805 | Negligible |
| 0.09 | 0.810 | 0.05 to 0.10 |
| 0.22 | 0.780 | ... |
| 0.46 | 0.760 | ... |
| 0.71 | 0.755 | 0.8 |
| 0.95 | 0.748 | ... |
| 1.20 | 0.750 | 6.0 |

**Solvents.** Since solvents other than ethanol are often necessary or desirable, several have been tried (Figure 4). A solution of the ferric reagent and ester sample in isopropyl alcohol is in every way comparable to ethanol. A benzene solution of the sample may be used with the remainder of reagents as in the procedure written above. Dioxane, if properly purified, will probably be a suitable solvent. Methylene chloride–ethanol mixtures and petroleum ether have also been used successfully. Diethyl ether has been used by Thompson (17) after extensive purification. Presumably other ethers and higher alcohols would be useful as solvents for the esters if purified. Water solutions of esters may be analyzed but at a slight loss in sensitivity, perhaps because of a competition between water and the hydroxamic acid

for the ferric ion. The same solvent should always be used for the calibration curve as for the determination.

**Spectrophotometric Data.** The absorption maxima and molar absorptivities obtained with a wide variety of esters are shown in Table III. The ferric complexes of most aliphatic hydroxamic acids have their absorption maxima at 530 m$\mu$. The ferric hydroxamate complexes of aromatic esters have broad absorption maxima at 550 to 560 m$\mu$. Ferric hydroxamate complexes of esters of acids containing conjugated double bonds or more than one carboxyl group may have maxima at slightly different wave lengths. The molar absorptivities are essentially the same for esters of the same acid and are additive for esters of polyhydroxyl alcohols. Esters of dicarboxylic acids have approximately twice the absorptivity found for similar monocarboxylic acids. Esters of resin acids form no color at all, perhaps owing to too weak hydrolysis conditions, allowing other esters to be determined when present in admixture.
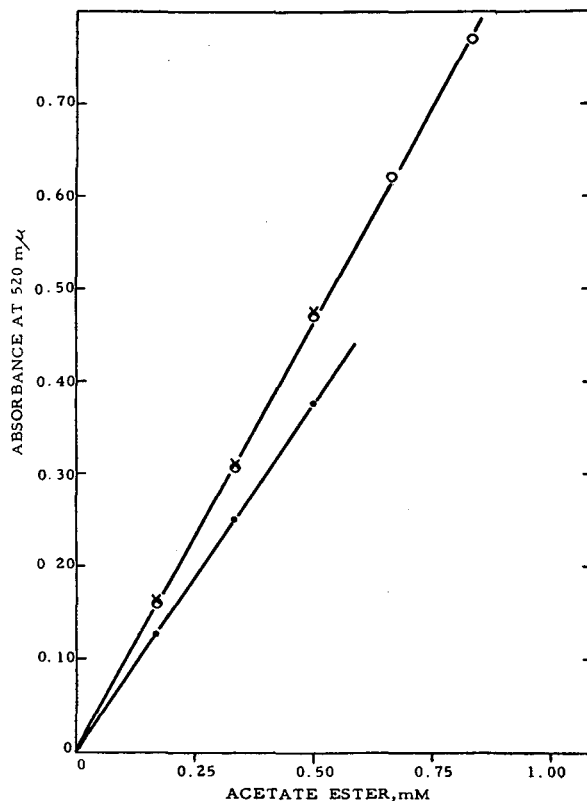


**Figure 3. Effect of hydrogen ion concentration on sensitivity of acetate ester determination**

Reaction time, 30 minutes
Ferric ion concentration, 2.1m$M$
Hydrogen ion concentrations
  ○  0.3$M$
  ✕  0.6$M$
  ●  1.0$M$

**Determination of Anhydrides in Presence of Esters.** Anhydrides of carboxylic acids also react in a manner similar to esters under the alkaline conditions discussed, forming 1 mole of hydroxamic acid per mole of anhydride which reacts. A method for the selective determination of anhydrides was developed based on the use of neutral hydroxylamine to form the hydroxamic acid. This reaction is similar in principle to one recently described for determining anhydrides in the presence of esters using a standard solution of morpholine as a base, and measuring the amount of morpholine remaining after the amide has been formed (10). The weak base morpholine, or in this case hydroxylamine,

is strong enough to react with the anhydride yet no reaction occurs with the ester. About 65% of the color which is obtained under alkaline conditions is developed using a 10-minute reflux time with the neutral reagent. The effect of appreciably increasing the reflux time was not investigated. It is assumed but not proved that the incomplete reaction of the anhydride is due to a competing reaction with the methanol which is present as the solvent for the hydroxylamine. Of the esters tested only phenolic esters, peroxyesters, lactones, and formates react. It has been reported that esters of electronegatively substituted acids such as the chlorinated acetic acids also react with hydroxylamine under neutral conditions (15). By applying the neutral reagent to mixtures of anhydrides and esters, or lactones and esters, a quantitative determination of the anhydride or lactone content is possible. Separate calibration curves must be used for the anhydride or lactone under basic and neutral conditions (Figure 5). Several representative analyses of mixtures are presented in Table IV. Both the precision and accuracy are reasonably good when it is considered that most such applications will be made to minor constituents.
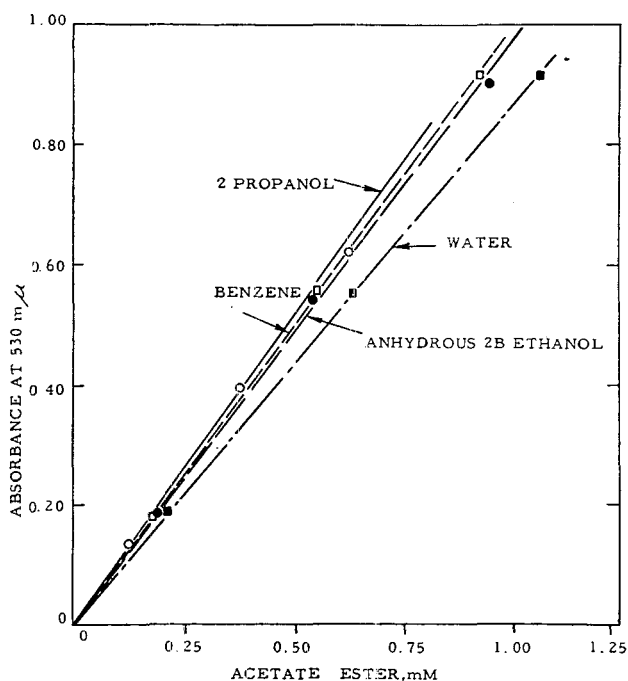


**Figure 4. Effect of ester solvent on ferric hydroxamate complex**

**Interferences.** Acids, most amides, and nitriles do not interfere with these procedures. The hydroxylaminolysis conditions are not severe enough to cause reaction of the latter two classes of compounds. Acid chlorides, of course, react in both procedures. High concentrations of carbonyls react with the hydroxylamine, probably necessitating the use of a higher hydroxylamine concentration. Transition elements such as copper, nickel, and vanadium react with hydroxamic acids to form colored chelate compounds which would interfere. It is possible that vanadium might be substituted advantageously for iron in the color development portion of the above procedure (3). Ions which complex ferric iron such as chloride, tartrate, acetic acid, and water may appreciably affect the intensity of the color in both the ester and anhydride methods.

**Extensions of Method.** Presumably this ester method could be used for the determination of amides and nitriles by using a
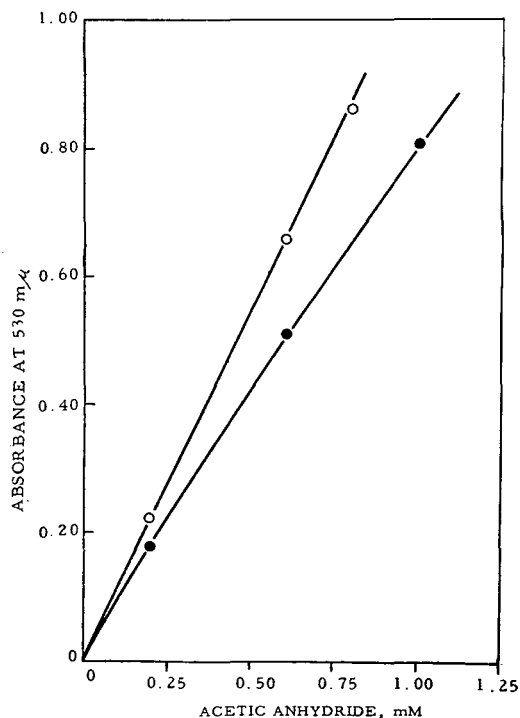


**Figure 5. Acetic anhydride in dry benzene calibration curves**

● Neutral reagent
○ Alkaline reagent

**Table III. Molar Absorptivities of Ferric Hydroxamates Formed from Different Esters**

| Esters | Wave Length, mμ | Molar Absorptivity, × $10^3$ |
|---|---|---|
| Ethyl formate | 520 | 1.06 |
| Ethyl acetate | 530 | 1.10 |
| n-Butyl acetate | 530 | 1.06 |
| n-Amyl acetate | 530 | 1.05 |
| Phenyl acetate (impure) | 530 | 0.99 |
| Triacetin | 530 | 3.33 |
| Ethyl propionate | 530 | 1.02 |
| γ-Butyrolactone | 530 | 1.11 |
| Methyl n-butyrate | 530 | 1.06 |
| n-Butyl n-butyrate | 530 | 1.05 |
| n-Amyl n-butyrate | 530 | 0.91 |
| Dimethyl malonate | 520 | 1.72 |
| Dimethyl maleate | 520 | 1.53 |
| Dimethyl adipate | 530 | 2.04 |
| Pentaerythritol tetracaproate | 530 | 3.89 |
| Methyl oleate | 530 | 1.00 |
| Methyl benzoate | 550 | 1.13 |
| n-Butyl benzoate | 550 | 1.08 |
| Benzyl benzoate | 550 | 1.13 |
| Methyl p-toluate | 550 | 0.94 |
| Dimethyl o-phthalate | 540 | 1.48 |
| Dimethyl isophthalate | 540 | 2.42 |
| Dimethyl terephthalate | 540 | 2.37 |

**Table IV. Mixture Analyses**

| No. | Anhydride Added, mg. | Anhydride Found, mg. | Anhydride Recovery, % | Ester Added, mg. | Ester Found, mg. | Ester Recovery, % |
|---|---|---|---|---|---|---|
| | ACETIC ANHYDRIDE-n-BUTYL ACETATE | | | | | |
| 1 | 3.10 | 3.17 | 102 | 2.80 | 2.85 | 102 |
| | 3.10 | 3.20 | 103 | 2.80 | 2.80 | 102 |
| | TOLUIC ANHYDRIDE-METHYL p-TOLUATE | | | | | |
| 1 | 2.00 | 1.85 | 93 | 8.10 | .. | .. |
| 2 | 6.00 | 5.90 | 98 | 4.05 | .. | .. |
| 3 | 2.37 | 2.25 | 95 | 5.87 | 5.82 | 99 |
| | 2.37 | 2.13 | 90 | 5.87 | 5.94 | 101 |
| 4 | 3.01 | 2.95 | 98 | 5.06 | 5.30 | 105 |
| | 3.01 | 3.00 | 100 | 5.06 | 5.25 | 104 |

higher boiling solvent such as propylene glycol as suggested by Soloway and Lipschitz (16). By using an excess of acetic anhydride substituted hydroxylamines may also be determined spectrophotometrically. The sensitivity of the method possibly may be increased by using the ultraviolet absorption spectra of the ferric–hydroxamic acid complexes.

Other compounds which may be converted to hydroxamic acids and possibly determined by their ferric hydroxamate colors are sulfonic acids, aldehydes, nitro compounds, and isocyanates (13, 18).

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# Ion Exchange Separation of Rhodium and Iridium

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The existing methods for the separation of rhodium and iridium are inadequate in terms of time and labor for the analytical chemist. The separation presented is fast, simple, and economical, and is based upon the behavior of the thiourea complexes of rhodium(III) and iridium(IV) toward a cation exchange resin. The iridium(IV)-thiourea complex behaves as an anion and passes through the column, whereas the rhodium(III)-thiourea complex behaves as a cation and is retained on the column. The rhodium(III)-thiourea complex is subsequently eluted with 6M hydrochloric acid at 74° C. The separation is quantitative and subsequent treatment yielded complete recovery of both metals as iridium(IV) chloride and rhodium(III) chloride.

THE separation of rhodium and iridium has long been a problem in the analysis of the platinum group metals. One of the earliest schemes utilized was that of Deville and Stas (3). The method was developed to determine iridium in alloys of platinum, but was applicable also for iridium in alloys of rhodium, palladium, gold, and copper. In this method the alloy was melted with about 10 times its weight of lead. This resulted in the formation of crystalline iridium and a new alloy whose constituents were lead and the metal originally alloyed with iridium. The lead alloy was soluble in acids, whereas the crystalline iridium was virtually insoluble even in aqua regia. This separation determined iridium only and was not used in the presence of metals that alloyed with the iridium under these conditions.

A quantitative separation of the two metals from one another was accomplished by Gilchrist (4) who utilized the selective reduction of rhodium to the metal by titanous chloride. This method was based on a qualitative procedure developed by Wada and Nakazono (8). The metallic rhodium was dissolved in boiling sulfuric acid and then reprecipitated. Two precipitations were usually sufficient to effect a quantitative separation. The titanium present was scavenged with cupferron and the iridium recovered by the hydrolytic precipitation of its hydrous oxide.

MacNevin and Tuthill (7) separated the two metals by electrol-

ysis using a controlled cathode potential. The method requires extensive instrumentation.

An attempt to separate rhodium and iridium utilizing an anion exchange resin was made by MacNevin and Crummett (6). They succeeded in recovering 95% of the rhodium but did not recover the iridium.

The separation presented here makes possible the complete recovery of rhodium and iridium as the chloro complexes.

### EXPERIMENTAL

**Apparatus.** A Beckman Model DU spectrophotometer with matching silica cells was used for the analysis of the rhodium and iridium solutions. The effluent from the ion exchange column was fractionated into 30-ml. fractions with a constant volume fraction collector. The ion exchange column used was fitted with a water jacket for temperature control.

**Reagents.** Rhodium(III) chloride and iridium(IV) chloride (A. D. Mackay, Inc.) were used for the preparation of stock solutions. The thiourea was procured from the Mallinckrodt Chemical Works. The resin utilized for the separation was Dowex 50W-X8, 50 to 100 mesh, obtained from Dow Chemical Co. All other chemicals used were reagent grade.

**Standardization of Solutions.** The stock solutions were prepared by dissolving the rhodium and iridium chlorides in 1M hydrochloric acid and were standardized according to the method worked out by Gilchrist (5). The metals were hydrolytically precipitated as their hydrous oxides and subsequently reduced to and weighed as the metals. The concentration of the iridium stock solution was 0.540 gram per liter and that of rhodium 1.11 grams per liter.

**Procedure.** The cation exchange resin, Dowex 50W-X8, was washed in successive operations as a batch process with water, 3M hydrochloric acid, and finally with water. The resin was then muddled into a slurry and packed into an ion exchange column with resin bed dimensions of 17.5 by 1.4 cm. The column was washed with 3M hydrochloric acid and the excess acid washed out with water.

The sample taken for separation consisted of 5.00-ml. aliquots of iridium stock solution and rhodium stock solution. Aqua regia was added and the resulting solution fumed to a moist residue on a steam bath. The residue was dissolved in 0.3M hydrochloric acid and heated on a steam bath. Solid thiourea was added while heating and the heating continued for 1 hour with sufficient additions of 0.3M hydrochloric acid to keep the volume constant.

The sample solution was cooled and passed through the cation exchange column at a flow rate of 2 to 3 ml. per minute. The first

colorless fractions of the effluent contained the iridium(IV)-thiourea complex. The rhodium(III)-thiourea complex was retained at the top of the column in a sharp reddish-orange zone. After the sample solution had completely passed through the column, the column was washed with 100 ml. of 3M hydrochloric acid at room temperature.

The elution of the rhodium-thiourea complex was carried out at 74° C. with 6M hydrochloric acid. These conditions were selected after a study of temperature and concentration effects upon elution.

**Temperature and Concentration Effects.** In investigating the temperature effect all conditions except temperature were kept constant. The elution of the rhodium-thiourea complex was carried out at three temperatures with the effluent fractionated and analyzed in each case. The results are shown in Figure 1.

In investigating the effect of concentration of the eluting agent, all factors other than the concentration of the eluting agent were kept constant. The results are shown in Figure 2.
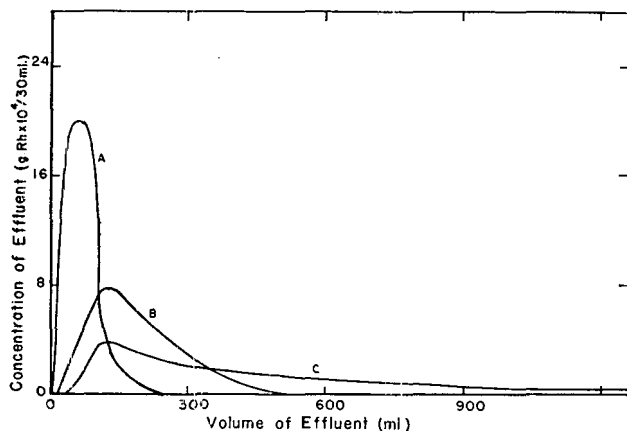


**Figure 1. Temperature dependency of rhodium-thiourea complex elution with 6M hydrochloric acid**

A. Elution at 74° C.
B. Elution at 50° C.
C. Elution at 25° C.

**Analytical Procedures.** The fractions collected were treated with aqua regia, boiled vigorously, and finally fumed to a moist residue. This step was repeated until the thiourea complexes were decomposed. The rhodium(III) chloride and iridium(IV) chloride were dissolved in 12M hydrochloric acid and fumed to dryness. The metal chlorides were redissolved in 1M hydrochloric acid and analyzed. The analysis of rhodium was carried out spectrophotometrically by the procedure of Ayres and Young (2). The iridium solutions were analyzed spectrophotometrically using the method developed by Ayres and Quick (1).

## DISCUSSION AND RESULTS

The results of six separations are shown in Table I.

**Table I. Quantitative Results of Separations**

|          | Mg. Taken | Mg. Recovered | % Recovered |
|----------|-----------|---------------|-------------|
| Iridium  | 2.70      | 2.70          | 100         |
|          | 2.70      | 2.70          | 100         |
|          | 2.70      | 2.70          | 100         |
|          | 2.70      | 2.70          | 100         |
|          | 2.70      | 2.70          | 100         |
|          | 2.70      | 2.70          | 100         |
| Rhodium  | 5.55      | 5.55          | 100         |
|          | 5.55      | 5.45          | 99.0        |
|          | 5.55      | 5.55          | 100         |
|          | 5.55      | 5.55          | 100         |
|          | 5.55      | 5.55          | 100         |
|          | 5.55      | 5.45          | 99.0        |

The preliminary treatment of the resin served two purposes. The first was to remove contaminants, chiefly iron, present in the resin. The second was to leave the resin in the hydrogen form. This treatment was unnecessary after the initial separation because all interfering contaminants were removed and the elution with 6M hydrochloric acid also served as the regeneration step. In packing the column glass wool plugs were used to support the resin.

The pretreatment of the sample converted the metal ions to the rhodium(III)- and iridium(IV)-chloro complexes. The time lag between addition of thiourea to the sample and introduction of the sample to the column was necessary in order to allow time for the complete formation of the complexes. The iridium-thiourea complex was formed rapidly but the rhodium-thiourea complex formed more slowly and was the limiting factor.

The flow rate during column operation was the maximum obtainable with the column dimensions and particle size used. The flow rate increased slightly as the temperature was raised but no attempt was made to regulate it.

The 100 ml. of 3M hydrochloric acid used to complete the elution of the iridium complex was necessary due to adsorption of the iridium-thiourea complex by the cation exchange resin. The amount of wash liquid increased with the amount of iridium present in the sample. During this process the rhodium-thiourea band on the column was broadened but not to a great extent. All elution curves reported in this paper involved the elution of the rhodium-thiourea complex after the treatment to displace the iridium-thiourea complex.

The high temperature elution of the rhodium-thiourea complex increased the efficiency of the process from the standpoint of both time and eluting agent. The temperature effect was marked and similar results with other systems have been noted. This, coupled with the use of 6M hydrochloric acid as eluting agent, presented a simple, yet rapid, elution.

An anion exchange resin may be used to effect the separation of the two metal complexes. However, the recovery of the iridium complex from the anion exchanger was more difficult than the recovery of the rhodium complex from the cation exchanger.
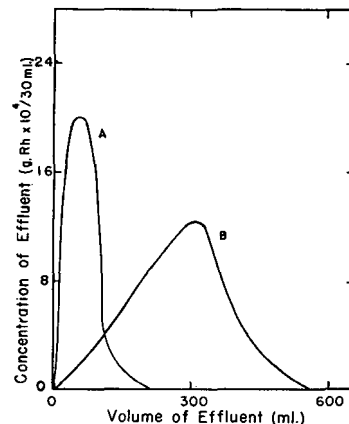


**Figure 2. Effect of concentration of eluting agent on elution of rhodium-thiourea complex at 74° C.**

A. Elution with 6M hydrochloric acid
B. Elution with 3M hydrochloric acid

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# Precision in X-Ray Emission Spectrography

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X-ray emission spectrography may be regarded as a random process, but only when operating conditions are ideal. Under such conditions, the standard deviation, background assumed negligible, should be predictable and equal to the square root of the mean number of counts, as it is in radioactivity. This conclusion was verified experimentally. When the actual standard deviation significantly exceeds the predicted, operating conditions are not ideal. This conclusion was verified experimentally for the case in which evaporation of the sample spoiled the alignment.

I N X-RAY emission spectrography, the intensities of the characteristic lines are generally so low that it is necessary to use a counter as detector, which is the usual procedure in studying radioactivity. In radioactivity, a truly random process (1, 5), the counts reaching the detector during successive identical counting intervals fluctuate according to a unique Gaussian distribution—that distribution for which the standard deviation is the square root of the mean. The purpose of this paper is to demonstrate that x-ray emission under ideal operating conditions also may be regarded as a random process, and to indicate how this affects the precision of analytical results. The background is assumed to be negligible throughout the study.

Under certain conditions, the counts made either on radioactive samples or in x-ray emission spectrography should obey a binomial or a Poisson distribution (1, 2). The analytical chemist normally is concerned only with conditions for which a Gaussian distribution should apply. The argument below can be extended to cover the other distributions as well.

The mathematical treatment of random fluctuations stems from probability theory (5), and has been verified experimentally for radioactivity (1, 4). The principal conclusion from this treatment appears below as translated to x-ray emission spectrography.

Assume that the spectrograph is operating satisfactorily—i.e., no assignable causes of variations are present—with a sample in place that has just given $N_1$ counts in $t$ seconds. Repeat this counting experiment $n - 1$ times to make available the series of counts $N_1 \ldots \ldots N_n$. Now, if $n$ and $N$ are large enough, the individual, counts $N_1 \ldots \ldots N_n$ lie on a Gaussian distribution of mean $\overline{N}$ and standard deviation $s_C = \sqrt{\overline{N}}$.

The experimental verification of this theoretical conclusion by Rutherford and Geiger (4) proved that radioactivity is a truly random process.

The theoretical conclusion that $s_C = \sqrt{\overline{N}}$ can be valid for radioactivity only if the total number, $N'_0$, of radioactive atoms in the sample remains virtually constant from the beginning of the first to the end of the last counting interval for a sample.

The conclusion $s_C = \sqrt{\overline{N}}$ is usually applied without comment or misgiving to x-ray emission spectrography, which differs from radioactivity in not being a spontaneous process. The conclusion thus applied can be strictly valid only when operating conditions are ideal. This statement becomes clear if the spectrograph is regarded as a means of maintaining in the sample a sensibly constant number $N_0$ of virtually identical excited atoms that emit the x-ray quanta being counted by the detector. The emission of such a quantum by any one of these $N_0$ atoms is a spontaneous process. This system of $N_0$ excited atoms maintained in the

sample by the action of the exciting beam is thus analogous to a radioactive sample. The conclusion $s_C = \sqrt{\overline{N}}$ therefore ought to hold in the former case, provided that always the same fraction of quanta emitted by the sample is counted by the detector.

The potential significance of this conclusion for the analytical chemist is clear from Figure 1, in which idealized distribution curves are given for the usual analytical method and for x-ray emission spectrography. In these frequency diagrams, the expected frequency for a given result is plotted as ordinate against the absolute value of the result, and standard deviations have been laid off along the abscissa from the means as origins.

For the usual (accurate) method, the mean $M$ is assumed identical with the true value, and observed errors are attributed to an indefinitely large number of small causes operating at random. The standard deviation, $s_A$, depends upon these small causes and may assume any value; mean and standard deviation are wholly independent, so that an infinite number of distribution curves, three being shown in Figure 1, is conceivable. But x-ray emission spectrography considered as a random process differs sharply from such a usual case. Under ideal conditions, the individual counts must lie upon the unique Gaussian curve for which the standard deviation is the square root of the mean. This unique Gaussian is a fluctuation curve, not an error curve in the strictest sense; there is no true value of $N$ as there is of $M$—there is only a most probable value $\overline{N}$.
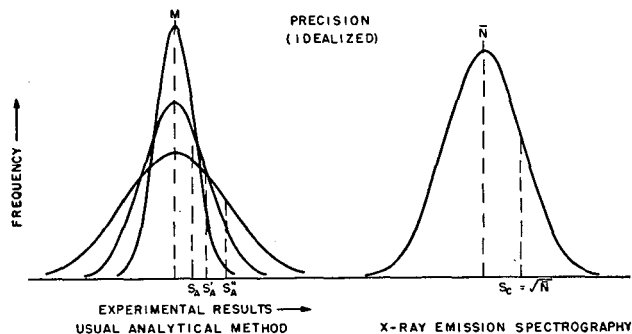


**Figure 1. Contrast between expected distributions of results for usual analytical method (error curves) and for x-ray emission spectrography (unique fluctuation curve) under idealized conditions**

Inasmuch as $s_C$ results from fluctuations that cannot be eliminated so long as quanta are counted, this standard deviation is the irreducible minimum for x-ray emission spectrography under ideal conditions. Not only is it a minimum but it is also a predictable minimum. When the actual standard deviation, $s_A$, significantly exceeds the predictable standard deviation, $s_C$, it is likely that errors resembling those the analytical chemist usually encounters are superimposed upon the random fluctuations.

This reasoning was tested experimentally as follows: With a tungsten sample in the spectrograph, the goniometer was adjusted until a counting rate near 100 counts per second was obtained. This counting rate is high enough to give a convenient counting interval and low enough to eliminate significant coincidence errors. The time required to reach 1024 counts was then

### Table I. Random Fluctuation in 393 Groups of N Counts Each

| Mid-point of interval[a] | 1100 | 1090 | 1080 | 1070 | 1060 | 1050 | 1040 | 1030 | 1020 | 1010 | 1000 | 990 | 980 | 970 | 960 | 950 | 940 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Frequency | 2 | 2 | 3 | 10 | 18 | 41 | 48 | 50 | 41 | 49 | 39 | 33 | 26 | 14 | 8 | 6 | 2 |

[a] Interval defined by $N$ (as listed) $\pm 5$ counts, except that lowest and highest intervals include all extreme values.

measured 393 times in succession. For each individual counting interval, the number of counts recorded for 10 seconds was calculated by simple proportion. (This arithmetic was without influence on the statistics.) In this way, a body of data was obtained for which $t = 1$ seconds; $n = 393$; and $\bar{N} = 1018$. These data are summarized in Table I according to frequency in the intervals identified by their mid-points.

The data of this table are plotted in Figure 2 about the Gaussian curve for which the standard deviation is the square root of the mean. The data of Rutherford and Geiger, which were obtained by counting alpha particles, are plotted about the same curve. In Figure 2, both sets of data fit the Gaussian about equally well.
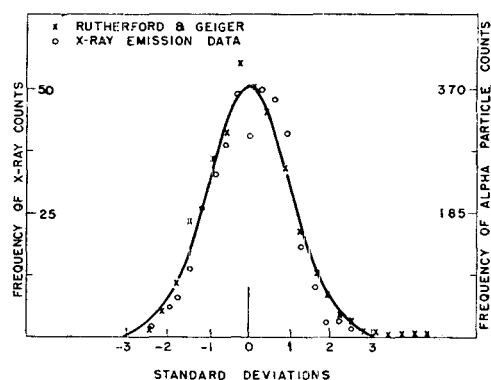


**Figure 2. Both x-ray emission spectrography and radioactivity conform to unique Gaussian curve based on $\bar{N}$ alone**

In both cases the fit is good enough so that there is little point in trying to guess the causes of the deviations. The conclusion is that the fluctuation treatment developed for radioactivity applies to an x-ray emission analysis being carried out under the best conditions.

#### PREDICTABLE STANDARD DEVIATION

A comparison of predictable and actual standard deviations can be valuable to the analytical chemist as an index to the operating conditions of his spectrograph. For Table I,

Predictable standard deviation =

$$s_C = \sqrt{\bar{N}} = 31.9 \text{ counts} \quad (1)$$

and for

Actual standard deviation =

$$s_A = \sqrt{\Sigma_i(N_i - \bar{N})^2/n - 1} = 29.5 \text{ counts} \quad (2)$$

Therefore,

$$s_A \cong s_C \quad (3)$$

which means that operating conditions for the spectrograph were satisfactory inasmuch as $s_C$ is the irreducible minimum standard deviation. It was encouraging to discover that good operating conditions could be maintained over the entire period (nearly a day) required to amass the data of Table I.

The small difference between $s_A$ and $s_C$ is probably attributable

to an error in $s_A$; this seems more likely because $s_C$ exceeds $s_A$. In general, $s_C$ is more precisely known.

When $s_A$ significantly exceeds $s_C$, there is an assignable cause of variation—in other words, operating conditions are not good. It is not possible to make the preceding statement quantitative, and this limits its usefulness when the differences between the standard deviations are not large.

One simple example of poor operating conditions is that of a volatile sample evaporating rapidly enough to change the optical path during the counting period. Owing to this change, the counts registered during $t$ seconds decreases with time if the spectrograph is not readjusted. This decrease is superimposed upon the random fluctuations discussed with the result that $s_A$ exceeds $s_C$.

A series of experiments paralleling those of Table I was carried out on an open cell containing toluene, with the goniometer set so as to give a counting rate near 100 counts per second at the mid-point of the series. The results are given in Table II.

### Table II. Effect of Drift Owing to Evaporation Superimposed on Random Fluctuations[a]

| Mid-point of interval[b] | 635 | 735 | 835 | 935 | 1035 | 1135 | 1235 | 1335 |
|---|---|---|---|---|---|---|---|---|
| Frequency | 28 | 31 | 28 | 26 | 33 | 34 | 16 | 4 |

[a] 200 groups of $N$ counts each; $t = 10$ seconds.
[b] Interval defined by $N$ (as listed) $\pm 50$ counts, except that lowest and highest intervals include all extreme values.

Inspection of Table II shows that the distribution is far from Gaussian. Calculation of the standard deviations (Equations 1 and 2) gives

$$s_A = 198 \text{ counts} \gg s_C = 31.9 \text{ counts} \quad (4)$$

Whenever $s_A$ thus exceeds $s_C$, operating conditions should be improved immediately. Further, when $S_A \gg s_C$, an increase in the number of counts is not necessarily reduced $s_A$.

The example of Table II illustrates one important difference between x-ray emission spectrography and the counting of radioactive disintegrations. In the former, the risk is greater that a disturbance of some sort increases the uncertainty of the result, especially if the counting period is prolonged. In x-ray absorption measurements, this risk is also present, and it has been reduced by using the comparative method (6).

Finally, precision is predictable in x-ray emission spectrography when Equation 3 is obeyed. Under these conditions, confidence limits may indeed be used with confidence (3).

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# Detection of Pentachlorophenol in Treated Wood

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Pentachlorophenol in treated wood was detected by formation of crystal violet (hexamethylpararosaniline chloride). Sections of treated wood were exposed to chlorine dioxide and sprayed with a 1% solution of crystal violet leuco base, $p,p',p''$-methylidynetris($N,N$-dimethylaniline), in xylene–Skellysolve E. Pentachlorophenol was converted to chloranil (tetrachloro-$p$-quinone), which oxidized the leuco base to crystal violet. During color development, oxidation by air was avoided by the use of an enclosure filled with nitrogen or carbon dioxide gas. The minimum pentachlorophenol concentration detectable in treated Ponderosa pine sapwood was estimated as 0.022%.

THE determination of penetration of pentrachlorophenol solution in treated wood generally has not been simple because of the essentially colorless nature of the compound and the small quantities in which it is found. When the solvents are dark-colored, penetration can be determined by observation of the darkened wood tissue. Oil-soluble dyes have been used for intensifying the color of preservative solutions, and fluorescent additives for detection in ultraviolet light. Powdered dyes have been used to impart color to the penetrated tissue of wood treated with a preservative solution of low volatility ($4$). These methods are based on the assumption that pentachlorophenol penetration is the same as that of other components of the solution. Determination of penetration of a clean, paintable type of formulation without a fluorescent additive must be through detection of pentachlorophenol itself.
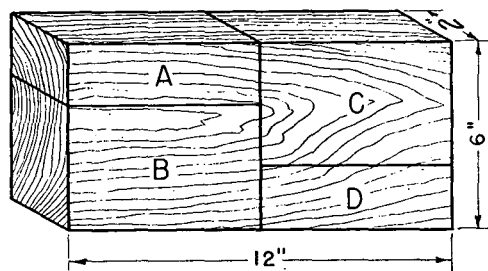


Figure 1. Pattern for cutting timber into block specimens

Pentachlorophenol forms colored salts with iron, nickel, cobalt, copper, etc., the dark red-purple color of copper pentachlorophenate is probably most easily detectable. An alcoholic solution of cupric acetate sprayed on the surface of treated wood will readily form copper pentachlorophenate, but the color formation is not discernible except when the pentachlorophenol content is above 1%.

Pentachlorophenol is oxidized by nitric acid to a yellow-red mixture of tetrachloro-$o$- and -$p$-quinones ($1$, $2$). When this reaction is carried out on wood, the wood substance itself is also darkened by nitric acid, which renders the similarly colored quinones undetectable except at higher concentrations.

Sandermann and Jonas ($3$) oxidized pentachlorophenol in treated wood to chloranil (tetrachloro-$p$-quinone) by exposing it to chlorine dioxide, and obtained the violet-blue color of methyl violet by subsequent spraying with a benzine solution of $N,N$-dimethylaniline. $N,N$-Dimethylaniline can also be converted to methyl violet by iodine, cupric salts, etc., as shown by Wichelhaus ($5$). The sensitivity of the Sandermann and Jonas method was found by this laboratory to be approximately 0.6% with respect to pentachlorophenol concentration in wood. Although this method provides a more sensitive detection than that by the copper pentachlorophenate method, it leaves much to be desired.
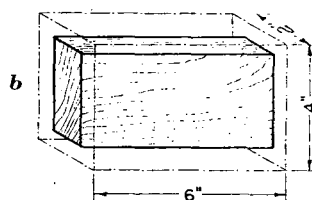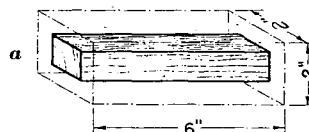


Figure 2

a. Derivation of blocks $A'$ and $D'$ from blocks $A$ and $D$
b. Derivation of blocks $B'$ and $C'$ from blocks $B$ and $C$

## THEORETICAL CONSIDERATION

In wood preserving industries, preservative absorption or retention has usually been expressed in terms of pounds per cubic foot of wood. Since the preservative solution is usually not uniformly distributed throughout each piece, actual preservative concentration in some parts is lower than the average preservative content. For example, in a piece of timber treated to a retention of 6 pounds of 5% pentachlorophenol solution per cubic foot of wood, the over-all preservative content is 0.96% of pentachlorophenol by weight (based on wood with a specific gravity of 0.5). If this timber is treated by one of the commonly used methods, the preservative concentration of the outer zone will exceed 0.96%, while that of the inner zone will be considerably lower, such as 0.1% or even less. To determine the extent of penetration to these low levels, a sensitive test is required.

The detailed reactions involved during the conversion of $N,N$-dimethylaniline by chloranil to methyl violet are not fully understood. It has been postulated that a methyl group in one $N,N$-
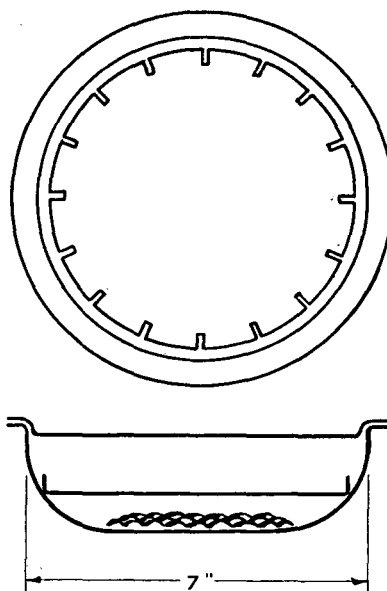


Figure 3. Apparatus for exposing wood sections to chlorine dioxide

dimethylaniline molecule is oxidized to formaldehyde, which condenses with the resulting N-methylaniline and two molecules of unaltered N,N-dimethylaniline to form pentamethylpara-rosaniline (the carbinol base of methyl violet).

The dyes methyl violet and crystal violet (hexamethyl-pararosaniline chloride) are converted by alkali to carbinol bases, which can be reduced to colorless leuco bases. These reactions are reversible. Therefore, it seemed probable that the use of

Figure 4. Nitrogen chamber used during color development

a leuco base instead of N,N-dimethylaniline as a color-forming reagent would produce three times as many moles of dyestuff per mole of chloranil, according to the following equations:

(1)

N,N-dimethyl-aniline        chlo-ranil        2H₂O
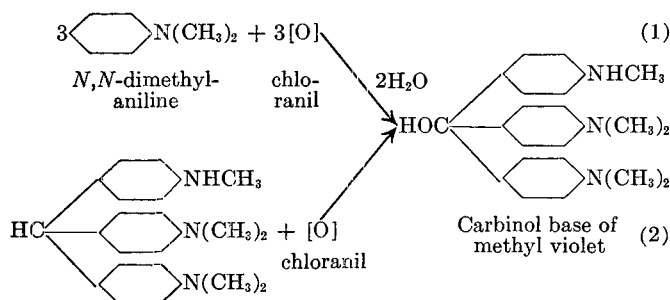
Leuco base of methyl violet

Since the leuco base of methyl violet was not readily available, whereas that of crystal violet, 4,4′,4″-methylidynetris(N,N-dimethylaniline), could be obtained from chemical supply companies (Eastman Kodak Co. No. 3651), the latter compound was chosen for study.

<div align="center">EXPERIMENTAL</div>

To determine the sensitivity of the color reaction, it was necessary to develop a method of treating and sampling wood so that the pentachlorophenol concentration at the limit of detection could be accurately estimated. Preliminary experiments included analysis of treated wood sections on which pentachlorophenol was barely detectable by the color reaction. Such analytical samples were taken from the interior of commercially treated lumber of large cross section (4 X 4 inch lumber or larger). The detection and analyses were also made on several specimens treated in the laboratory with both pressure and nonpressure methods. It was found that by dip-treating the ends of carefully duplicated specimens cut from even-grained, clear-grade Ponderosa pine sapwood, the duplicates could be treated to the same preservative retention with practically the same concentration gradient from the dipped ends.

Wood blocks were cut from a piece of timber according to the pattern shown in Figure 1; blocks A and D were 2 X 2 X 6 inches and blocks B and C, 2 X 4 X 6 inches. Although the A–D pair differed from the B–C pair in cross-sectional area, they were essentially duplicates with respect to preservative concentration gradient. The uniformity of the grain was readily verified because of the cutting pattern and the pairing of specimens in detection and analysis. The blocks for analysis (B and C) were cut larger than A and D. Cross sections of the larger blocks provided sufficient pentachlorophenol for accurate quantitative determination at the limit detectable by the color reaction. Very thin cross-sectional slices (2 mm.) were taken to assure an accurate

estimate of pentachlorophenol in each increment of the block. In treating, the blocks were suspended vertically at the same level, and a 2.5% solution of technical pentachlorophenol in 1 to 1 (by volume) xylene–mineral spirits was brought into contact with the lower ends so that they were immersed to a depth of 0.25 inch for 3 minutes. After the treatment, the blocks were left suspended in air for 5 days to condition to equilibrium. The conditioned blocks were then "shelled" by sawing off 0.5-inch slabs from all four sides, leaving blocks A′, B′, C′, and D′ as shown in Figure 2 (solid lines).

The crystal violet color reaction was carried out on longitudinally split sections of blocks A′ and D′ (not shown in drawing). The wood sections were exposed for 30 minutes to chlorine dioxide gas, generated by the action of glacial acetic acid on sodium chlorite. This treatment was made in a glass casserole (household type) approximately 7 inches in diameter (Figure 3). Ten grams of sodium chlorite was placed in the casserole and sufficient water was added to form a paste, to which approximately 8 ml. of glacial acetic acid was added. The reactants were then covered with a 6-inch Petri dish, the bottom of which had been perforated at 1-inch intervals around the circumference. The perforated dish, which rested on the curved sides of the casserole about 1 inch from the bottom, also served as a support for wood specimens.

After treatment with chlorine dioxide, the wood specimens were aired for 0.5 hour before they were sprayed with a 1% solution of crystal violet leuco base in 1 to 1 (by volume) xylene–Skelly-solve E. Immediately after spraying, the specimens were placed in a nitrogen chamber (Figure 4). A violet color soon developed, starting at the dipped ends, and within 5 minutes the color formation extended to its maximum length of approximately 2.3 inches. The pentachlorophenol concentration at this point was found to be approximately 0.022% by analysis as shown later.

A slight coloration eventually developed on the remainder of the specimens, and even on untreated, unoxidized controls. This might have been caused by traces of air that were already present in the wood.
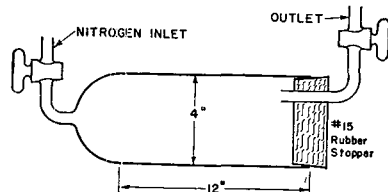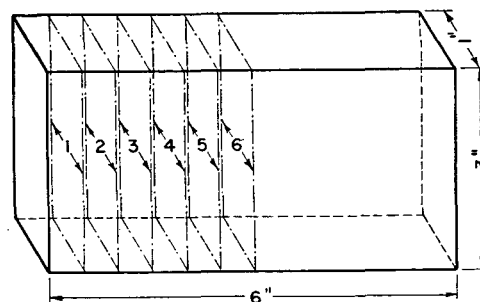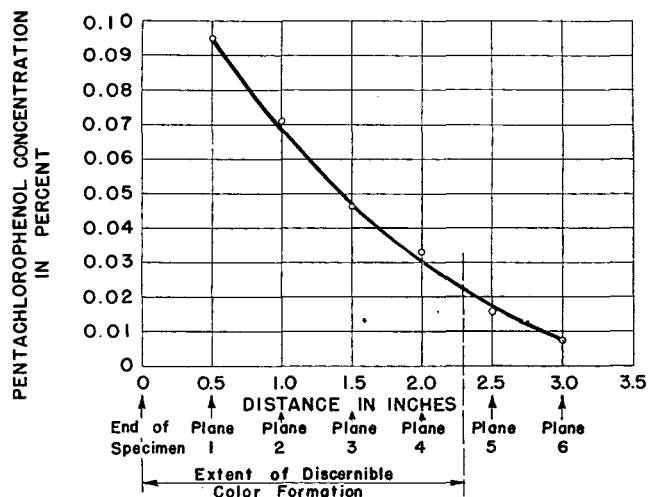
Figure 5. Zoning of blocks B′ and C′

Figure 6. Distribution and detectable limit of pentachlorophenol in wood

The pentachlorophenol concentration in planes 1, 2, 3, 4, 5, and 6 of blocks $B'$ and $C'$ (Figure 5) was estimated by analysis of a composite of adjacent cross sections, 2 mm. thick, on either side of these planes. The two sections for each analysis were pulverized together in a Kenmore liquidizer and digested in 2.5% sodium hydroxide. Pentachlorophenol was recovered by acidification with hydrochloric acid and steam distillation. The isolated pentachlorophenol was determined by the nitric acid oxidation method (2). The results are given in Figure 6, in which the extent of penetration, as revealed by the color of crystal violet, is also shown. From these results it is estimated that 0.022% is the limit of pentachlorophenol concentration detectable by the crystal violet method in the kind of wood studied.

## DISCUSSION

Clear-grade Ponderosa pine sapwood was used in the experiment because of the uniformity of its grain and color. It was found that, for all practical purposes, the degree of sensitivity estimated is also applicable to other species of light-colored wood, and to other types of pentachlorophenol formulations. Obviously, the sensitivity would be considerably lower for dark-colored wood. It is also conceivable that extractives of some species of wood and some ingredients used in pentachlorophenol formulations might interfere with the color reaction.

It was found that the degree of sensitivity determined was reliable only when applied to freshly exposed surfaces of wood sections. The amount of pentachlorophenol, and consequently of chloranil, to react with the leuco base is very small in the vicinity of the detectable limit, probably in the order of a fraction of a microgram per square millimeter. Volatilization of pentachlorophenol is likely to cause a significant reduction of the surface concentration upon prolonged exposure.

Carbon dioxide from a cylinder or from sublimation of dry ice is a satisfactory substitute for nitrogen as a means of excluding air during color development.

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# Chromatographic 2,4-Dinitrophenylhydrazone Method for Determination of Allethrin

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A method is described for the determination of allethrin based on its conversion to the 2,4-dinitrophenylhydrazone derivative, chromatographic analysis of the derivative on silicic acid, and gravimetric or colorimetric determination of the main band.

IN 1949 Schechter, Green, and LaForge (23) discovered a method of synthesizing esters of the pyrethrin type. One of these esters, dl-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one esterified with a mixture of cis- and trans-dl-chrysanthemummonocarboxylic acids and called allethrin (20), is now being produced commercially at the rate of about 50,000 pounds per year (22). It now costs about $32 per pound.
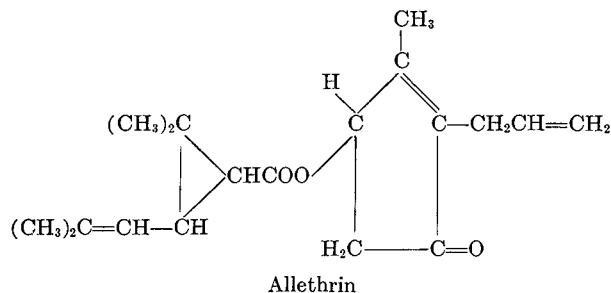


Allethrin

As with all new chemicals, it is important to have a number of methods of analysis available. The development of analytical methods for allethrin has been difficult because of the complexity of the molecule. Allethrin is similar to the pyrethrins in structure, and some of the methods used for the analysis of pyrethrins, such as the AOAC (1) and Seil (27) methods, can be adapted for certain purposes. However, the accuracy and precision of these methods leave something to be desired in spite of attempts to improve them in recent years.

The hydrogenolysis method (12) for pyrethrins has also been adapted to the analysis of allethrin (4). It was used for the assay of allethrin until it was discovered that chrysanthemummonocarboxylic anhydride was present in allethrin and that an adequate correction for the anhydride could not be made by this method. Hogsett, Kacy, and Johnson (10) have recently proposed a method in which allethrin reacts with ethylenediamine. This splits the ester and forms an equivalent of chrysanthemummonocarboxylic acid, which is then titrated in a nonaqueous medium. At present it is the preferred procedure for the assay of allethrin, for it has the advantages of reproducibility and adaptability to the running of many samples in a reasonable time; in addition a number of acidic impurities found in technical samples are determined. It does, however, have certain disadvantages, such as the number and quantity of solvents and standard solutions required, some difficulty with the end points found with dark-colored samples, and the need for large samples (5.3 to 8.6 grams per single analysis).

In some of these methods it is necessary to make separate determinations of acidic impurities, such as chrysanthemummonocarboxylic acid, its anhydride, and its acid chloride, in order to correct for the presence of these materials.

A number of other methods have been proposed—some chromatographic (15, 29), some colorimetric (2, 5, 13, 14, 26), and others modifications of existing methods. A polarographic method has been presented by the Japanese workers Yamada, Sato, and Iwata (30) and an infrared method by Freeman (6).

The authors' method is based on the formation of the 2,4-dinitrophenylhydrazone derivative of allethrin, its chromatographic separation on a silicic acid column, and gravimetric determination of the main band thus separated. A colorimetric determination can also be used for smaller amounts where less accuracy is tolerable. Since the derivative is colored, its progress down the column can be readily observed. Some impurities, such as allethrolone, form bands which easily separate from the

main band. Although Lord *et al.* (*15*) and Oiwa *et al.* (*17*) mention dinitrophenylhydrazones of pyrethrins, no method has been developed nor have adequate conditions been given for the formation of the dinitrophenylhydrazones of pyrethrins or allethrin; and their formation in high yield is a critical matter. The authors' 2,4-dinitrophenylhydrazone procedure has certain advantageous features, such as utilization of the entire allethrin molecule rather than just the acid formed on cleavage, the formation of a colored derivative which can be chromatographed, the use of small samples, and the fact that acidic impurities need not be determined separately. Although it may not be so precise or accurate as the ethylenediamine method, it is proposed because additional methods based on different types of reactions are often needed as a check on one another or for further development and modification in special applications which may arise.

Since this article was written, an article by Moore (*16*) has described the determination of allethrin and pyrethrins by a 2,4-dinitrophenylhydrazone procedure. The authors' method differs from Moore's in the choice of adsorbent, solvent, reaction conditions, extraction procedure, and in other details. With the reaction conditions of the present method, the dinitrophenylhydrazone of the whole allethrin molecule is formed, whereas with Moore's method the ester is split and a derivative of allethrolone is formed, probably identical with the dinitrophenylhydrazone from band *B* (see Discussion).

### REAGENTS AND APPARATUS

2,4-Dinitrophenylhydrazine (Eastman Kodak Co.), extracted twice with hot benzene and air-dried or recrystallized.

Hydrochloric acid, concentrated (12*N*).

Benzene, redistilled from dinitrophenylhydrazine. One hundred milliliters should contain less than 1 mg. of nonvolatile matter.

Skellysolve B or hexane, redistilled from dinitrophenylhydrazine. One hundred milliliters should contain less than 1 mg. of nonvolatile matter.

Silicic acid, chromatographic grade (Mallinckrodt Chemical Works or equivalent).

Hyflo Supercel (Johns-Manville Co.).

Ether, anhydrous and free from aldehydes and ketones.

Methanol, anhydrous, redistilled from dinitrophenylhydrazine.

Eluting solvent, mixture of 500 ml. of benzene, 500 ml. of Skellysolve B, and 30 ml. of ether.

Separatory funnels, 250-ml., lubricated with the starch-glycerol lubricant described by Herrington and Starr (*9*).

Volumetric flasks, 100 ml.

Erlenmeyer flasks, 125 ml.

Vacuum oven.

Absorbent cotton, extracted with acetone, dried in a vacuum oven at 80° C.

Cylindrical funnels, Gooch crucible holders, similar to Ace Glass Co. No. 6145 except that the internal dimensions are ca. 13 mm. in diameter × 60 mm.

Chromatograph tubes, 25 mm. in diameter × 400 mm. with fritted disk (Scientific Glass Apparatus Co. No. J-1665-1, or equivalent).

### GRAVIMETRIC PROCEDURE

**Preparation of the Derivative.** About 600 mg. (0.002 mole) of the sample is weighed into a 125-ml. Erlenmeyer flask. The reagent solution is prepared by refluxing 600 mg. (0.003 mole) of 2,4-dinitrophenylhydrazine with 50 ml. of anhydrous methanol containing 0.25 ml. of concentrated hydrochloric acid (accurately measured). The reagent solution is cooled to room temperature (26° to 28° C.), poured onto the sample, and allowed to stand at room temperature for 2.5 hours, with occasional swirling. If the sample is not miscible with the methanol (when kerosine is present), the reaction should be run in a stoppered flask on a shaking machine for 2.5 hours.

**Extraction of the Derivative.** The methanol solution is poured into a 250-ml. separatory funnel and diluted with 60 ml. of cold water (5° C.). Twenty milliliters of benzene are used to rinse the flask into the funnel, which is shaken vigorously; the contents are then allowed to settle. The reaction flask is rinsed with 15 ml. of benzene into a second funnel, then with successive 10-ml. portions into a third and a fourth funnel. The aqueous layer from the first funnel is successively extracted in the second, third, and fourth funnels and then discarded. It may take about 5
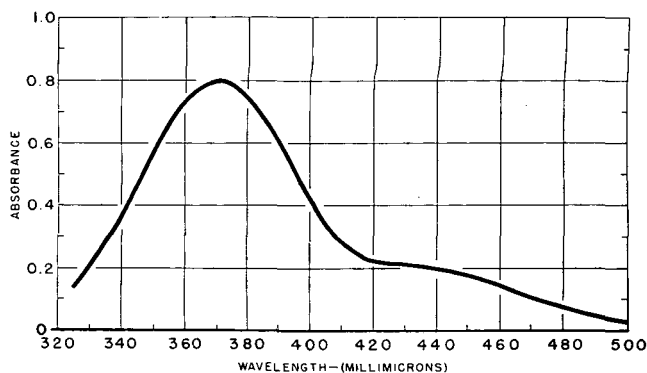


**Figure 1. Absorption spectrum of α-*dl*-*trans*-allethrin dinitrophenylhydrazone**

1.44 mg. per 100 ml.

minutes for the layers to separate reasonably well in the first funnel before the aqueous layer can be drained.

The first three portions of benzene are combined in the first funnel, any water present is drained off, and the benzene is then filtered into the third funnel through a small plug of cotton contained in a cylindrical funnel. This procedure removes most of the excess dinitrophenylhydrazine, which is rather insoluble in benzene. The fourth portion of benzene is used to rinse the two empty funnels, the rinsings also being filtered through the cotton plug into the third funnel. Two more 5-ml. rinses are passed successively through each empty funnel and finally through the cotton into the third funnel. The stems of the funnels may be washed off with a fine stream of benzene from a medicine dropper, the tip of which has been drawn down to a capillary.

The filtered, combined extract is washed twice by shaking with 15 ml. of saturated sodium chloride solution. After the last traces of sodium chloride solution have been drained off, the extract is filtered through a clean plug of cotton in a cylindrical funnel directly into a 100-ml. volumetric flask. Several small washings are employed to rinse the funnel and its stem into the filter funnel. The solution is then diluted up to the mark on the volumetric flask and shaken.

**Blank Determination.** A blank determination is made in the same way with the same lots of reagents and solvents, except that no sample is used.

**Preparation of Column.** The adsorbent consists of 2 parts by weight of silicic acid and 1 part of Hyflo Super-Cel. No activation seemed to be necessary with the lots that the authors used. About 20 to 22 grams of this mixture are suspended in sufficient eluting solvent to make a thin slurry. After thorough mixing (a magnetic stirrer was used), this slurry is rapidly poured into the chromatographic column. Air bubbles may be removed by stirring with a long glass rod. The walls of the column are washed down with a few milliliters of fresh solvent, and the column is compacted by gentle tapping and the application of moderate pressure from a nitrogen cylinder. The compacted adsorbent is generally 120 mm. high and is ready for the introduction of the sample.

**Introduction of Sample onto Column.** A 20.0-ml. aliquot is taken from the volumetric flask, drained into a 30-ml. beaker, and evaporated on the steam bath until the volume is 4 to 5 ml. Evaporation is hastened and boiling of the liquid is prevented by blowing a gentle stream of clean air across the surface of the liquid. Excessive heating should be avoided, since benzene-insoluble resins may be formed, particularly if the sample is allowed to go to dryness and is heated for any length of time.

When most of the suspending liquid has drained through the column, to within 5 mm. of the top of the adsorbent, the sample is quantitatively transferred to the column. This is done by using an elongated form of medicine dropper with a bent, constricted tip and allowing the introduced liquid to drain down the column wall. One milliliter of the mixed solvent is used to rinse the beaker and the dropper. The liquid is now allowed to flow into the adsorbent assisted by gentle pressure from a compressed-air line or, preferably, a nitrogen tank. Two or three more small rinsings will transfer all the material, each portion being allowed to enter the adsorbent before the next portion is added. The column is filled with eluting liquid, and if desired a device may be attached to apply a gas pressure of 25 to 30 cm. of water. Such a device may be made from a T-tube with the vertical arm submerged 25 to 30 cm. into water in a cylinder. Care should be

taken never to allow the column to run dry either during the introduction of the sample or subsequently, since channeling will ruin the determination.

**Elution of Bands.** After about 40 ml. of eluting liquid have drained from the column, the first band will approach the outlet. Three or more bands will now be seen, and the bulk of the material appears in the first band (band *A*). This band is usually rather wide and diffuse and may seem to be heterogeneous, possibly owing to the formation of syn and anti forms of the dinitrophenylhydrazones as noted by Gordon (7). The rear of the band is generally more intense than the front portion. The entire band will be swept from the column in about 60 to 80 ml. of solvent, which are collected in a weighed 125-ml. Erlenmeyer flask. Some of the tailings are collected in the same flask until band *B* approaches the outlet. The space between the bands on the column appears to be pale yellow, but the eluate between the main bands contains a negligible weight of material. The tip of the column is finally rinsed into the flask with a few drops of solvent.

The receiving flask is heated on the steam bath until the solvent has evaporated, evaporation being hastened by blowing a gentle stream of air across the mouth of the flask. Drying is completed by heating the flask in a vacuum oven at 75° C. under a vacuum of about 60 to 70 cm. An hour in the oven is generally sufficient to bring the flask to constant weight. After cooling to room temperature the flask is weighed.

A blank determination in which the same lots of reagents are used should be run through the whole procedure. The solvent is collected in the same amount at about the same elution volume as in a run with sample present. The per cent of allethrin may be calculated as follows:

$$\% \text{ allethrin} = \frac{313.4 \text{ (weight of band } A - \text{weight of blank)}}{\text{weight of original sample}}$$

Table I shows the results obtained with this method on seven allethrin samples compared with those given by the ethylenediamine method. The two methods agree well, except in the case of sample C. No reason can be offered at present for this discrepancy.

**Table I. Comparison of 2,4-Dinitrophenylhydrazone and Ethylenediamine Methods of Analysis of Allethrin Samples**

| Sample[a] | 2,4-Dinitrophenylhydrazone Method | | Ethylenediamine Method[b] |
|---|---|---|---|
| | Allethrin, % | Average, % | Allethrin, % |
| A | 97.8, 98.1 | 98.0 | 98.2 |
| B | 88.5, 87.0 | 87.7 | 90.3 |
| C | 84.1, 84.9, 84.6 | 84.5 | 92.6 |
| D | 80.8, 81.6 | 81.2 | 78.6 |
| E | 90.0, 91.2 | 90.6 | 91.8 |
| F | 91.9, 91.2 | 91.5 | 92.2 |
| G | 87.3, 85.2, 84.6 | 85.7 | 88.8 |

[a] All samples are commercial lots except A, which is crystalline α-dl-trans isomer of allethrin (25).
[b] Average of two or more determinations.

Ordinarily it is not necessary to collect band *B*, which is the next band to come off the column after band *A*, or band *C*, which is a slow-moving band (or group of bands) at the top of the column. The properties of bands *B* and *C* are described in the discussion.

### COLORIMETRIC PROCEDURE

The colorimetric procedure utilizes the same principles as the gravimetric procedure.

As it is necessary to measure the hydrochloric acid accurately, the dinitrophenylhydrazine reagent solution is made up in a quantity sufficient for two samples. This solution contains 250 mg. of dinitrophenylhydrazine dissolved in 28 ml. of anhydrous methanol containing 0.10 ml. of concentrated hydrochloric acid (accurately measured). A quantity of sample calculated to contain about 100 mg. of allethrin is mixed with 14 ml. of this reagent solution, and put on a shaking machine for 2.5 hours. The derivative is then extracted as described under the gravimetric procedure, except that larger amounts of benzene may be employed. After making up to a volume of 200 ml., an aliquot of 5.00 ml. is withdrawn and delivered to the top of the chromatographic column. Band *A* is collected in the normal manner and is made up to 100 ml. in a volumetric flask. Further dilution may

be necessary to reach a range where the color intensity may be determined in a photometer. In this laboratory a Beckman Model B spectrophotometer was used. The measurements were made at a wave length of 375 mμ—the calibration mark on the wave-length dial closest to the broad peak (373 mμ) of the curve for purified α-dl-trans-allethrin dinitrophenylhydrazone shown in Figure 1. Beer's law is followed very well with absorbance values up to 0.6, as shown with a standard curve obtained by carrying α-dl-trans-allethrin through the procedure.

**Table II. Colorimetric Analysis of Allethrin Samples**

| Sample | Allethrin, % | Average, % |
|---|---|---|
| A | 101, 108, 109 | 104 |
| B | 80, 89, 96 | 88 |
| C | 83, 90, 93 | 89 |
| D | 70, 83, 93 | 82 |
| E | 86, 96, 96 | 93 |
| F | 90, 101, 105 | 99 |
| G | 85, 87, 95 | 89 |
| H | 4.4, 5.0, 6.8 | 5.4 |
| I | 1.3, 1.8, 2.1 | 1.7 |

Table II presents some results obtained by the colorimetric method applied to the same samples which had been used for the gravimetric determinations. Sample H is an aerosol formulation which contains 4.2% of allethrin, 13.33% of DDT, 33.33% of Velsicol AR-50 (a methylated naphthalene), and 49.13% of Deobase (a purified kerosine). Sample I is another aerosol formulation which contains 1.06% of allethrin, 13.93% of Deobase, 51.67% of AR-50, 13.33% of methoxychlor, 13.33% of lethane 384 (butoxy thiocyanodiethyl ether), and 6.66% of the synergist MGK 264. While the results with the colorimetric method are probably accurate to only about ±10%, and results with formulations are not as good as desired, it is believed that this method may serve as a rough check on certain formulations where other methods have failed.

The authors have found that the sensitivity of the colorimetric procedure can be increased at least twofold by adding alcoholic alkali to the final 2,4-dinitrophenylhydrazone solutions, and taking readings at 435 mμ (18).

### DISCUSSION

This method depends on the formation of the 2,4-dinitrophenylhydrazone derivative of the entire allethrin molecule. After extraction the derivative is chromatographed on a column of silicic acid and Hyflo Super-Cel. In general, the scheme is similar to that of Roberts and Green (21) and Gordon and coworkers (7) for aldehydes and ketones with certain modifications. Because the dinitrophenylhydrazones of allethrin are rather insoluble in petroleum ether as used by Gordon, it was necessary to change the solvent. This was done by adding an equal volume of benzene to the petroleum ether even though the sharpness of the bands was decreased. The benzene–petroleum ether mixture appears to be more sensitive to the addition of small percentages of ethyl ether than does petroleum ether. A number of solvent combinations with various amounts of benzene, petroleum ether, and ether were tested before the solvent mixture was selected. Alumina was tried and found to be too strong an adsorbent. Other adsorbents such as talc seemed to be too weak.

**Advantages.** The gravimetric procedure may be employed in almost any laboratory with comparatively simple equipment and reagents. Smaller samples are used than in the existing methods. It is not necessary to determine or correct for acidic impurities, as in other methods. The color of the original sample is not of much consequence; dark samples occasionally cause difficulties in observing the end points in some other methods. The colorimetric procedure allows the method to be applied to very small amounts, although at the loss of accuracy and precision. The whole molecule forms the 2,4-dinitrophenylhydrazone instead of being cleaved as in other methods. The color of the derivative

allows the chromatographic separation to be followed easily by eye.

**Disadvantages.** All the solvents and some of the reagents have to be carefully purified. Large quantities of solvents are used, and they are not easily recoverable for re-use. The extraction of the derivative requires considerable care. The method is subject to certain interferences.

**Interferences.** Certain interferences from the solvents and reagents employed must be guarded against, lest the blank determinations be too high. It is desirable to recrystallize the 2,4-dinitrophenylhydrazine reagent or to extract it several times with hot benzene in order to remove impurities. The solvents employed in this procedure usually contained traces of aldehydes or ketones; for this reason they are refluxed with 2,4-dinitrophenylhydrazine and then distilled from this reagent before use. For the extractions a stopcock grease insoluble in benzene must be employed.

Good recoveries may be obtained from allethrin mixtures containing up to 5% of each of the three acidic contaminants appearing in technical allethrin—namely, chrysanthemummonocarboxylic acid, its anhydride, and its acid chloride.

A number of common insecticides and synergists will interfere in the gravimetric procedure unless certain modifications are made. For example, DDT and piperonyl butoxide will interfere unless the procedure is modified as follows:

The silicic acid is suspended in 1 to 1 benzene–Skellysolve B and, after the dinitrophenylhydrazone is introduced on the column, the benzene–Skellysolve B mixture (no ether) is used for elution until the interfering substances leave the column. The dinitrophenylhydrazones move very slowly. After the DDT or piperonyl butoxide has been eluted, the usual 1 to 1 benzene–Skellysolve B solvent containing 3% of ether is used for elution and the derivative is collected in the normal manner. In some cases columns of silicic acid twice as long as those described in the gravimetric procedure were found to be advantageous when working with interfering materials.

The synergist piperonyl cyclonene forms a derivative with 2,4-dinitrophenylhydrazine. When chromatographed, it smears the entire column, seriously interfering with the determination of allethrin.

As might be expected, the pyrethrins also interfere. When carried through the described procedure five bands are formed. Methoxyl determinations indicate that a clean separation of pyrethrin I and pyrethrin II (as dinitrophenylhydrazones) is not attained. However, it is possible that this procedure might be adaptable to the analysis of pyrethrum.

The new insecticide cyclethrin (8) also interferes. Preliminary tests indicate that the percentage of cyclethrin in the commercial product may be determined by the proposed method.

**Size of Sample.** The size of sample was chosen to allow convenient extraction and gravimetric operations. Although the chromatographic column may be somewhat overloaded by the amount of dinitrophenylhydrazone employed, adequate separations of the bands were obtainable under the described conditions. No doubt the gravimetric procedure can be adapted to smaller samples if desired. Also a change in the amounts of aliquots would allow the use of smaller samples.

**Concentration of Acid and Time of Reaction.** The effect of varying the concentration of acid and the reaction time on the formation of the dinitrophenylhydrazone of a technical sample of allethrin is shown in Figure 2. It can be seen that 0.25 ml. of concentrated hydrochloric acid and a reaction time of 2.5 hours are best. If too little acid is used, the reaction is too slow and it is difficult to get enough of the 2,4-dinitrophenylhydrazine in solution. On the other hand, if the acid concentration is too high, the derivative that is formed is cleaved or decomposed and the yield decreases with time.

**Experiments with Crystalline α-dl-trans-Allethrin.** Early in the investigation it was found that when the pure crystalline α-dl-trans isomer of allethrin [first suggested as a standard by Schechter et al. (24, 25)] was used, excellent gravimetric recoveries of the dinitrophenylhydrazone could be obtained by the method of Iddles and Jackson (11). About 3.5 grams of the crystalline allethrin were dissolved in 200 ml. of anhydrous ethyl alcohol. The reagent solution was prepared by dissolving 5.0 grams of 2,4-dinitrophenylhydrazine in 190 ml. of methanol and adding 10 ml. of concentrated hydrochloric acid. Equal volumes (10.0 ml.) of the two solutions were mixed and allowed to stand for various lengths of time. The crystalline precipitate was then filtered on a weighed fritted-glass funnel, washed with 2N hydrochloric acid, and dried at 105° C. The results are shown in Table III. It is apparent that prolonged standing reduces the amount of derivative recovered, a fact discussed by Iddles and Jackson (11). The crystals are orange-colored plates melting about 118–120° C. When recrystallized from 95% ethyl alcohol, the melting point was 129.0–129.5° C.

**Table III.    Precipitation of α-dl-trans-Allethrin Dinitrophenylhydrazone**

| Reaction Time, Hours | Recovery, % |
|---|---|
| 1 | 96.4 |
| 2 | 100.2 |
| 3 | 98.1 |
| 4 | 98.9 |
| 20 | 97.7 |

**Analysis.** Calculated for $C_{25}H_{30}N_4O_6$, carbon, 62.23; hydrogen 6.27. Found, carbon 61.26; hydrogen 6.18.

The absorption curve of this 2,4-dinitrophenylhydrazone in benzene–Skellysolve B (1 to 1) to which 3% by volume of ether was added is given in Figure 1.

**Experiments with Commercial Allethrin.** The method employed with the crystalline α-dl-trans isomer of allethrin could not be employed with commercial samples. Although the crystalline isomer readily gave a crystalline precipitate of the 2,4-dinitrophenylhydrazone, all the commercial samples, even those of over 90% purity, gave gummy, noncrystalline 2,4-dinitrophenylhydrazone derivatives, which could not be filtered and washed. This is due to the fact that allethrin is a mixture of four racemic stereoisomers, and the mixture of the dinitrophenylhydrazones does not crystallize readily. For these reasons extraction and chromatography were resorted to in order to eliminate by-products and interferences. A certain degree of success was attained with the partition chromatographic technique of Ramsey and Patterson (19). Very sharp, clear bands were formed, but the low solubility of the dinitrophenylhydrazones in the mobile solvent (hexane saturated with nitromethane) severely limited the amount of material that could be introduced onto the column. Therefore, the straight chromatography on silicic acid described in this article was developed.

**Band B.** After the main allethrin 2,4-dinitrophenylhydrazone band (band A) has been eluted, a second band (band B), consisting of a 2,4-dinitrophenylhydrazone, comes off the column. This compound occurs to some extent on all the columns, except when pure α-dl-trans-allethrin dinitrophenylhydrazone is chromatographed. With the amounts of technical allethrin described in the gravimetric procedure, the band B eluted from the column ordinarily weighs about 5 to 15 mg. Although it can be considered to be a breakdown product formed from allethrin dinitrophenylhydrazone by the action of acid and although the amount increases with the time of standing in acid solution, it is still possible that some is formed from a slight impurity in technical allethrin samples, since the amounts formed from technical allethrin are greater than those from the pure α-dl-trans isomer.

To test the effect of prolonged standing, an experiment was conducted in which allethrin dinitrophenylhydrazone was formed

as described above in the regular procedure, except that the reaction mixture was allowed to stand 4 days instead of 2.5 hours. This sample, which ordinarily produced $A$ and $B$ bands containing about 180 and 12 mg., respectively, after 2.5 hours, now produced bands of 69 and 101 mg. This proves that the amount of compound responsible for band $B$ formed from allethrin dinitrophenylhydrazone increases on long standing in the presence of acid.

When the material from band $B$ was isolated and recrystallized from ethyl alcohol, the red crystals had a melting point of 143–144° C.

Analysis. Calculated for $C_{15}H_{16}N_4O_5$, carbon, 54.20; hydrogen, 4.85; nitrogen, 16.87. Found, carbon, 54.91; hydrogen, 5.19; nitrogen, 15.80.

The compound of band $B$ has the same empirical formula as allethrolone dinitrophenylhydrazone (see below), but is not identical with it. The possibility that it might be a pyrazoline type of compound seems to be excluded by Bredt's rule (3). Therefore, the nature of its isomerism with allethrolone dinitrophenylhydrazone remains unexplained.
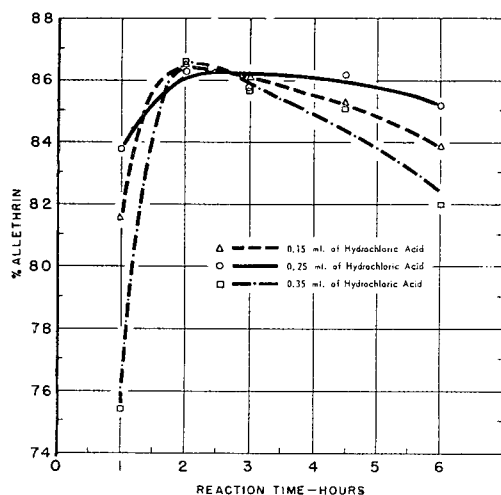


**Figure 2. Effect of varying concentration of acid and reaction time on determination of allethrin**

That this compound is not formed via the acid hydrolysis of allethrin first and then reaction with dinitrophenylhydrazine is shown by an experiment in which allethrin was allowed to stand in dilute methanolic hydrochloric acid for several days before the addition of 2,4-dinitrophenylhydrazine. It was found that band $B$ was close to the normal weight (only 18.1 mg.), band $A$ was somewhat lighter than usual (154 mg.), and band $C$ (largely allethrolone dinitrophenylhydrazone) was much heavier (55 mg.).

Allethrolone 2,4-dinitrophenylhydrazone is isomeric with the compound from band $B$ and may be converted into this compound under certain conditions. When allethrolone dinitrophenylhydrazone is prepared from allethrolone under very mild conditions (no heat and only a small excess of acid), the normally expected derivative is formed. After recrystallization from ethyl alcohol the orange crystals melt at 205–207° C. (decomposition).

Analysis. Calculated for $C_{15}H_{16}N_4O_5$, carbon, 54.20; hydrogen, 4.85; nitrogen, 16.87. Found, carbon, 54.57; hydrogen, 4.81; nitrogen, 16.83.

This derivative is strongly adsorbed on the chromatographic column and stays near the top of the column during an analysis. However, if an attempt is made to prepare the same deriva-

tive by the method of Shriner and Fuson (28), in which the solution is heated, the final product is red and melts at 143–144° C. after recrystallization.

Analysis. Calculated for $C_{15}H_{16}N_4O_5$: carbon, 54.20; hydrogen, 4.85; nitrogen, 16.87. Found, carbon, 55.43; hydrogen, 5.31; nitrogen, 16.54.

The mixture melting point with the compound from band $B$ shows no depression, an indication that the compounds are identical. Heating the high-melting allethrolone dinitrophenylhydrazone (melting point 205–207° C.) in an oven at 110° C. for several hours did not change its melting point, but it can be converted into the lower melting compound of band $B$ by refluxing allethrolone dinitrophenylhydrazone for a half hour in ethyl alcohol containing some concentrated hydrochloric acid.

Allethrolone dinitrophenylhydrazone is practically unaffected by standing in dilute methanolic hydrochloric acid for 4 days at room temperature. Allethrolone itself also remains virtually unchanged under these conditions, since subsequent addition of 2,4-dinitrophenylhydrazine gave the normal allethrolone dinitrophenylhydrazone.

**Band $C$.** Band $C$ consists mainly of allethrolone dinitrophenylhydrazone plus excess 2,4-dinitrophenylhydrazine, which is extracted during the benzene extraction. Both of these compounds move very slowly in the chromatographic procedure described above.

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# Determination of Nordihydroguaiaretic Acid in Creosote Bush

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**An analytical procedure was needed to determine the nordihydroguaiaretic acid present in creosote bush. In the procedure developed, the machine-threshed, fresh creosote bush is extracted with *n*-butyl ether, which is then recovered by steam distillation. The impurities are separated by means of their substantial insolubility in boiling water or in boiling 0.25% acetic acid-0.1% sodium bisulfite solution and by adsorption on activated carbon from a boiling aqueous 0.25% acetic acid-0.1% sodium bisulfite solution of the crude or impure nordihydroguaiaretic acid. The pure nordihydroguaiaretic acid is insoluble in the cool, carbonized, modified aqueous solvent and is filtered, washed, dried, and weighed. The nordihydroguaiaretic acid content of two shipments of fresh machine-threshed creosote bush was found to be 1.84 and 1.31%.**

THE determination of nordihydroguaiaretic acid in creosote bush has been difficult because colorimetric and optical methods are not specific for this acid. The work reported here is the quantitative separation of the impurities by chemical means and the consequent determination of nordihydroguaiaretic acid (NDGA) by weighing the purified chemical as such.

Lundberg and Halvorson (9) devised procedures for the determination of nordihydroguaiaretic acid and other phenolic antioxidants in fats. Their data were based on the color reaction between an antioxidant phenol, including nordihydroguaiaretic acid, and a modified iron-bipyridine reagent, first described by Emmerie and Engel (2). Although the reaction is not specific for nordihydroguaiaretic acid, it is useful in determining only one phenolic antioxidant, the identity of which is known.

An effort was made to use ultraviolet spectrophotometry for the determination of nordihydroguaiaretic acid. Almost identical absorption spectra, with absorption maximum close to 2800 A., were given by each of the following: twice recrystallized nordihydroguaiaretic acid, melting point 186–187° C.; impure nordihydroguaiaretic acid, melting point 181–185° C.; impure nordihydroguaiaretic acid, melting point 177–182° C., and red amorphous impurities, melting point 62–75° C. (adsorbed on an eluted from activated carbon). Structural similarity of nordihydroguaiaretic acid to the impurities associated with and separated from the acid may account for this phenomenon. However, this explanation is not certain.

In the method given here, nordihydroguaiaretic acid is separated from the impurities associated with it in the primary extract and in the crude nordihydroguaiaretic acid, and the pure acid is recovered quantitatively. A modest departure from the proximate assay method previously described (10) is the use of *n*-butyl ether (boiling point 141–142° C.) rather than isopropyl ether as the solvent in contact with the threshed creosote bush and to extract the nordihydroguaiaretic acid. The commercial, and other, extraction methods for nordihydroguaiaretic acid are disclosed in seven patents (1, 3–8).

The crude nordihydroguaiaretic acid, melting point 172–180° C. (and similar melting ranges) is separated as described earlier (10) by boiling distilled-water extractions of the primary extract creosote or tar residue. The crude acid is purified by recrystallization from boiling 0.25% acetic acid-0.1% sodium bisulfite solution. The impurities are removed from the impure nordihydroguaiaretic acid solution by adsorption on activated

carbon when the boiling solution, saturated with respect to nordihydroguaiaretic acid, is filtered through a prepared bed of the activated carbon. Waller (12) used 10 to 15% and 36% acetic acid as solvents for the nordihydroguaiaretic acid. In this laboratory, a number of the hydroxy acids (lactic, citric, salicylic, tartaric, and phenol) were tried as recrystallization solvent for the nordihydroguaiaretic acid. However, acetic acid proved to be better. Numerous activated carbons were investigated.

The primary tar or creosote can be extracted directly, at 100° C., with the recrystallization solvent (0.25% acetic acid-0.1% sodium bisulfite solution), and then the resulting boiling crude nordihydroguaiaretic acid solution can be brought in contact with activated carbon and filtered. The hot crude nordihydroguaiaretic acid solution can be stirred mechanically with the activated carbon or the boiling crude nordihydroguaiaretic acid solution can be filtered through a carbon bed. Both pressure- and vacuum-type filtration equipment were used in this work. However, the direct purification is too cumbersome to accomplish quantitative recovery in the laboratory.

In the direct method, the primary tar, freed of *n*-butyl ether, is extracted by mechanically stirring with boiling 0.25% acetic acid-0.1% sodium bisulfite solution rather than with boiling distilled water. The pure nordihydroguaiaretic acid, melting point 185–187° C., precipitates from the carbon-treated, filtered, cooled, 0.25% acetic acid-0.1% sodium bisulfite extract of the primary tar. Distilled water extraction of the primary tar yields only the crude nordihydroguaiaretic acid, melting at 172° to 180° C. and similar melting points.

The direct method of purification was part of a proposal by the author for the production of nordihydroguaiaretic acid. However, the procedure was criticized because of the excessively large volumes of hot aqueous impure nordihydroguaiaretic acid solution which would be pumped through the filter press (11).

## PROCEDURE

The sample, 1250 grams of fresh, green machine-threshed creosote bush, is extracted by two successive 12-liter portions of cool peroxide-free (sodium bisulfite-washed) *n*-butyl ether. The second solvent extraction gave assurance of complete recovery of the nordihydroguaiaretic acid. The sample is held in a fine-meshed, cylindrical copper basket, with twill cloth circles retaining the material at the top and bottom. After the sample is extracted and drained free of solvent, the solvent is recovered from the extract solution by steam distillation. The recovery of *n*-butyl ether is almost complete.

The brown tar residue, or creosote, fluid at 100° C. is extracted quantitatively by 10 or 11 successive 3-liter portions of boiling distilled water. The boiling extracts are filtered through cotton. Crude nordihydroguaiaretic acid fractions, melting at 172–180° C. and similar melting points, precipitate from the cool extracts. In order to make certain that the nordihydroguaiaretic acid has been recovered quantitatively, excess extractions are made from which no crude nordihydroguaiaretic acid precipitates. The crude acid is filtered and dried at 105° C.

The colloidal yellow filtrate contains nordihydroguaiaretic acid and impurities. The filtrate liquor is extracted quantitatively with successive 1-liter portions of peroxide-free *n*-butyl ether. Usually two, sometimes three, *n*-butyl ether extractions are required to take the phenols and impurities completely out of a 15- or 16-liter portion of the filtrate liquor. The extracted filtrate liquor gives negative tests for phenols with 5% ferric chloride and also with concentrated sodium hydroxide solution. The crude nordihydroguaiaretic acid is obtained from the *n*-butyl ether solution in the same manner in which the crude nordihydroguaiaretic acid is recovered from the first *n*-butyl ether extract of the creosote bush.

**Purification of Crude Nordihydroguaiaretic Acid.** The dry,

crude nordihydroguaiaretic acid, with the fractions of it mixed (melting point 167–179° C.), is purified by recrystallization from 16 to 18 liters of a boiling solution of 0.25% acetic acid and 0.1% sodium bisulfite in distilled water. The crude nordihydroguaiaretic acid is dissolved by adding 0.5- to 1.0-gram increments slowly with stirring to the boiling aqueous solvent. Small amounts of tarry or resinous-looking impurities are insoluble. The boiling, intensely yellow solution is filtered by vacuum through a hot bed of activated carbon (Darco G60) in a Büchner funnel. The impurities are adsorbed, and a fraction of the nordihydroguaiaretic acid is temporarily held by the activated carbon.

The pure nordihydroguaiaretic acid, melting point 185–187° C., completely precipitates from the cool carbonized filtrate, which is faintly colored. This acid is white.

The carbon is washed at once while very hot with successive 1-liter portions of boiling distilled water until the washed carbon is free of nordihydroguaiaretic acid—that is, until no nordihydroguaiaretic acid crystals form in the cooled carbon wash water. Excess washings of the carbon were made to ensure the quantitative recovery of the nordihydroguaiaretic acid. Five or six boiling water extractions of the carbon and insoluble impurities were usually needed. The nordihydroguaiaretic acid washed from the carbon is cream colored.

The boiling water extraction completely washes the nordihydroguaiaretic acid from the carbon. Extraction of the water-washed carbon by isopropyl ether or n-butyl ether or hot concentrated acid solution always failed to extract any nordihydroguaiaretic acid from the water-washed carbon beds.

The pure acid, melting point 185–187° C., is filtered, washed with distilled water, dried at 105° C., and weighed. The main yield of pure acid is collected and weighed separately. The carbon wash yield of nordihydroguaiaretic acid is treated similarly in order to separate the yield data.

The following are typical data for the recrystallization of the crude nordihydroguaiaretic acid.

| 0.25% Acetic–0.1% Bisulfite Solutions, Liters | Crude NDGA (M.P. 169–180° C.), Grams | NDGA (M.P. 185–187° C.) from Crude, Grams[a] Main | C | H₂O-Insol. Impurities Separated, Grams |
|---|---|---|---|---|
| 8 | 22.5 | 6.1 Total | 2.6 8.7 | 1.9 (orange-red) |

[a] 30-gram carbon bed used.

## DISCUSSION OF RESULTS

Crude nordihydroguaiaretic acid is obtained by hot distilled-water extraction of the brown primary tar or creosote residue after distillation of the n-butyl ether. The precipitated crude acid which is filtered, dried, and weighed is termed A in Table I. The crude acid, recovered quantitatively by means of suitable extraction of the filtrate liquor obtained from the filtration of crude nordihydroguaiaretic acid yield A, is classified under B.

### Table I. Yield of Nordihydroguaiaretic Acid from Creosote Bush[a]

| Machine-Threshed Creosote Bush | Crude NDGA A Grams | A M.p., °C. | Crude NDGA B Grams | B M.p., °C. | Recryst. NDGA, Grams Main | C | Rec. | Total | NDGA, % |
|---|---|---|---|---|---|---|---|---|---|
| Earlier shipment | 26.1 | 172–79 | 24.2 | 167–78 | 17.9 | 5.0 | 0.1 | 23.0 | 1.84 |
| Recent shipment |  |  |  |  |  |  |  |  |  |
| 1 | 18.6 | 175–81 | 15.1 | 171–79 | 10.9 | 5.0 | 0.1 | 16.0 | 1.28 |
| 2 | 24.0 | 172–80 | 21.0 | 169–77 | 11.6 | 5.7 | 0.0 | 17.3 | 1.38 |
| 3 | 28.1[b] | 134–78 | 14.7 | 168–80 | 10.7 | 4.9 | 0.2 | 15.8 | 1.26 |

[a] 1250-gram sample used.
[b] 0.03% NaCl added to increase precipitation of crude NDGA from colloidal condition.

The first extraction of the creosote bush sample by n-butyl ether was always quantitative. This is demonstrated from the fact that no nordihydroguaiaretic acid was ever obtained by means of the second n-butyl ether extraction of the creosote bush sample. The residual creosote or tar, obtained from the second n-butyl ether extraction, was extracted three times with 3-liter portions of boiling distilled water.

The nordihydroguaiaretic acid, melting point 185–187° C., was obtained by recrystallization of the mixed A and B fractions of crude nordihydroguaiaretic acid. The yield is classified

### Table II. Microdetermination of Carbon and Hydrogen

| NDGA Source[a] | Sample, Mg. | Found, % C | H |
|---|---|---|---|
| | Nordihydroguaiaretic Acid | | |
| Earlier shipment | 20.34 | 71.35 | 7.83 |
| | | 71.41[b] | 7.33[b] |
| Present shipment | | | |
| 1 | 25.36 | 71.43 | 7.79 |
| 2 | 21.57 | 71.36 | 7.62 |
| 3 | 27.30 | 71.57 | 7.62 |

Unrecrystallized Nordihydroguaiaretic Acid Tetraacetate

| | Sample, G. | NDGA Tetraacetate, G. Obtained | Theory | Dev. from Theory, Parts/1000 | C | H |
|---|---|---|---|---|---|---|
| Crude NDGA, twice recryst., m.p. 186–187° C. | 0.9790 | 1.5237 | 1.5234 | .... | 66.52 | 6.65 |
| | | | | | 66.37[c] | 6.43[c] |
| Earlier shipment | 1.0234 | 1.5870 | 1.5925 | −3.5 | 66.35 | 6.11 |
| Present shipment | | | | | | |
| 1 | 0.9889 | 1.5364 | 1.5388 | −1.6 | 66.88 | 6.75 |
| 2 | 0.9973 | 1.5474 | 1.5519 | −2.9 | 66.14 | 6.72 |
| 3 | 0.9914 | 1.5394 | 1.5427 | −2.1 | 66.46 | 6.66 |

[a] See Table I.
[b] Theory for $C_{18}H_{22}O_4$.
[c] Theory for $C_{26}H_{30}O_8$.

under several headings: main for the main yield and C for the carbon wash yield. Rec. indicates the quantity of the nordihydroguaiaretic acid obtained from quantitative extraction of the combined recrystallization filtrates from the main and C yields of nordihydroguaiaretic acid.

**Extraction of Recrystallization Filtrate.** The combined filtrate, after filtration of the nordihydroguaiaretic acid yield, was extracted by 1 liter of peroxide-free n-butyl ether. The first extraction was always complete and took out any nordihydroguaiaretic acid and associated impurities. After the n-butyl ether had been steam-distilled, the small orange-red residue, liquid at 100° C., was dissolved in 1 liter of boiling 0.25% acetic acid-0.1% sodium bisulfite solution. This boiling solution was filtered through a 20-gram bed of G60 Darco with vacuum. The carbon bed then was washed twice, each time by 1 liter of boiling distilled water. Any nordihydroguaiaretic acid which precipitated from the cool carbonized filtrates was filtered, dried, and weighed.

**Purity of Nordihydroguaiaretic Acid.** Microdeterminations of carbon and hydrogen were made on the nordihydroguaiaretic acid samples (Table II) and on the unrecrystallized nordihydroguaiaretic acid tetraacetate derived from each nordihydroguaiaretic acid yield. These data indicate a very high degree of purity for the nordihydroguaiaretic acid samples.

In the quantitative conversion of nordihydroguaiaretic acid to nordihydroguaiaretic acid tetraacetate, it was necessary to acetylate the nordihydroguaiaretic acid sample with excess acetyl chloride. Waller (12) employed acetic anhydride in first describing the preparation of nordihydroguaiaretic acid tetraacetate. However, acetic anhydride did not quantitatively convert catechol or nordihydroguaiaretic acid to the corresponding diacetate and tetraacetate.

The nordihydroguaiaretic acid tetraacetate was prepared in the following way.

The nordihydroguaiaretic acid sample was refluxed for 4 hours with 25 ml. of redistilled acetyl chloride in a glass esterification apparatus. Almost all of the excess acetyl chloride then was boiled off on a hot water bath. The cool reaction mixture was dissolved in 75 ml. of diethyl ether. The ether solution was washed in a separatory funnel twice with 40-ml. portions of distilled water, twice with 50-ml. portions of 1% sodium bicarbonate solution, and twice with 40-ml. portions of distilled water. The final water wash was free of any acidity or any bicarbonate

ion. The combined washings were re-extracted by two 25-ml. portions of ether. The latter ether extract (50 ml.) was washed with 25 ml. of distilled water, then with 10 ml. of distilled water. The combined ether extract was filtered through a small cotton pad (in a buret funnel) into a tared beaker. The cotton was washed carefully with 10-ml. portions of ether after most of the ether had been evaporated from the extract in the beaker. The nordihydroguaiaretic acid tetraacetate was then dried at 105° C. for a minimum of 2 hours, and held in a vacuum desiccator prior to microanalysis.

The molecular weight of nordihydroguaiaretic acid, $C_{18}H_{22}O_4$, is 302.36. The molecular weight of nordihydroguaiaretic acid tetraacetate, $C_{26}H_{30}O_8$, is 470.50. In theory, 1.000 gram of pure nordihydroguaiaretic acid yields 1.5561 grams of nordihydroguaiaretic acid tetraacetate.

### ACKNOWLEDGMENT

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# Infrared Spectrophotometric Determination of Allethrin

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An infrared spectrophotometric method has been developed for the determination of allethrin. The intense 5.81-micron band was selected for this study, and it was ascertained that only one of the four impurities known to be present in the insecticide interfered at the analytical wave length. Chrysanthemummonocarboxylic acid anhydride was shown to be present as a minor constituent in the samples investigated. The 5.56-micron band was utilized for determining the anhydride. Allethrolone has been determined by means of its 2.86-micron absorption maximum, and standard and differential techniques have been compared. cis- and trans-allethrins exhibit different absorptivities at 5.81 microns, and the relative amounts of the isomers were determined by their absorbances at 8.70 and 8.85 microns.

ALLETHRIN is a commercially available insecticide similar to the pyrethrins in action.
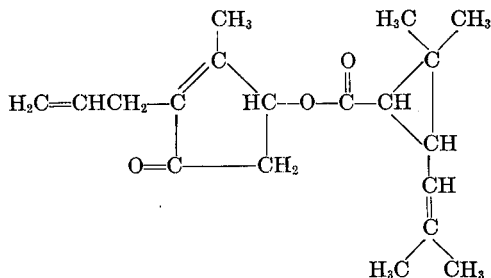


There are two methods at present employed in industry for the analysis of this substance, and both have certain disadvantages. The hydrogenolysis procedure of Schechter (*18*) fails to take into account one of the interfering impurities present in commercial allethrin (chrysanthemummonocarboxylic anhydride) and, in some instances, gives erratic results when the purity of the insecticide is less than 90%. The ethylenediamine method (*12*) requires daily standardization of solutions prior to their use, the

exact location of the end point is difficult to detect when off-color samples of allethrin are analyzed, and the reagents employed are rather expensive. Cueto and Dale (*3*) have published a colorimetric method for determining allethrin, and Oiwa, Shinohara, Takeshita, and Ohno (*16*) investigated the polarographic analysis of $\alpha$-*dl*-*trans*-allethrin. Harris (*11*) has developed a chromatographic method for the insecticide, and a spectrophotometric procedure has recently been reported (*14*).

While this article was being reviewed, a paper (*15*) discussed the determination of allethrin by weighing the chromatographed 2,4-dinitrophenylhydrazone. Green and Schechter (*8*) have developed a similar method.

Elliott (*4*) has recently published entomological data on the trans content of allethrin, and his average figure of 75% agrees well with that of the author. Elliott attempted to explain the reason for the radical difference between his results and those of Gersdorf and Mitlin (*7*) by stating that it might be due to "different reactive conditions and varying nonstoichiometric ratios of the reagents in the preparations of the esters." Schechter stated that the materials supplied to Gersdorf for his studies were prepared by the procedure outlined in his original paper (*17*). Therefore, some other reason must be sought to explain the divergency.

Crombie (*4*) recently found, by infrared spectrometry, that methyl chrysanthemumate contains 68% of the trans isomer. A short while later (*10*) Harper and Sleep observed that chrysanthemumonitrile contains 73% of this isomer. By means of a modified AOAC method (*1*), the quantity of *trans*-chrysanthemum-monocarboxylic acid present in the racemic acid was found to be nearly identical with that reported in this paper (*19*).

An infrared spectrophotometric method has been developed for determining allethrin, utilizing the intense 5.81-micron band (Figure 1). It was first necessary to examine the infrared spectra of the impurities occurring in commercial allethrin. Allethrolone (Figure 2), chrysanthemummonocarboxylic acid (Figure 3), its acid chloride (Figure 4), and its anhydride (Figure 5) were prepared, and it was ascertained quantitatively, by determining the absorption of purified allethrin containing known amounts of added impurities, that only allethrolone interfered at 5.81 microns in the concentrations encountered in the technical product.
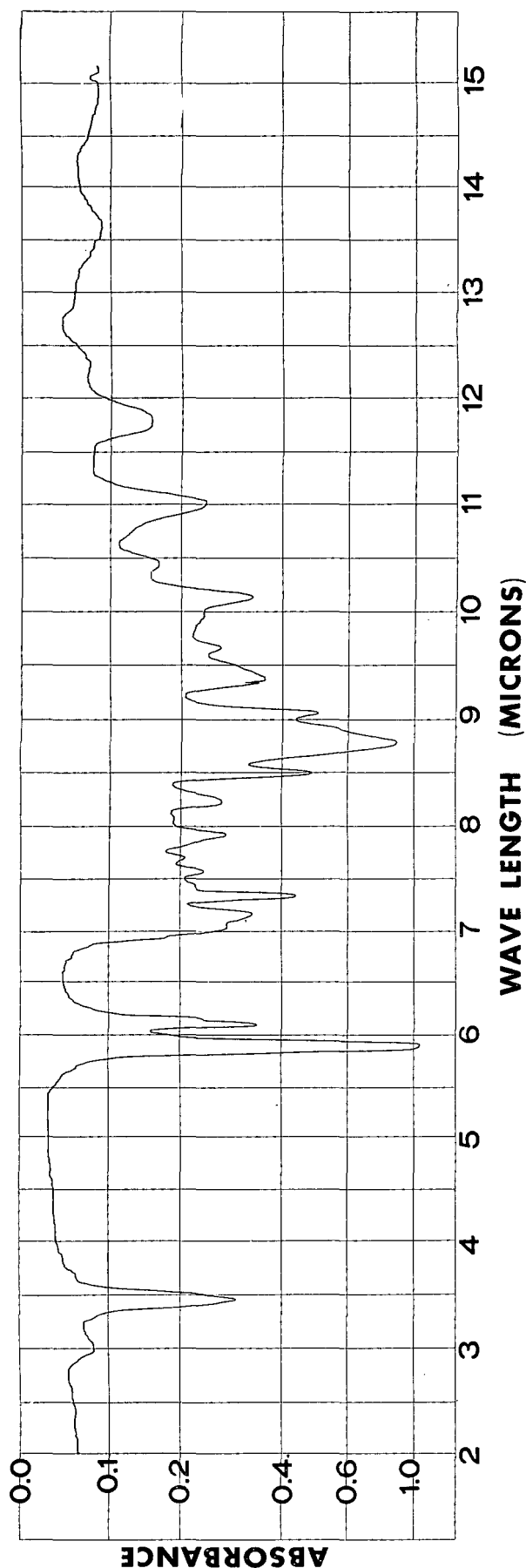
**Figure 1.** Infrared spectrum of allethrin (between salt plates)

The anhydride affects the absorbance of allethrin at this wave length in amounts exceeding 5%, and the largest quantity found in the insecticide samples studied was 3%.

Hogsett, Kacy, and Johnson (12) state that the presence of chrysanthemummonocarboxylic acid anhydride in most samples was established by chemical analysis and confirmed by infrared data, but present no actual experimental proof of its existence. The author has separated the anhydride from allethrin by partition chromatography, using nitromethane on silicic acid and n-hexane as the mobile solvent (6). Its identity was proved by comparison of its infrared spectrum with that of an authentic sample, in addition to chemical analysis.

The determination of chrysanthemummonocarboxylic acid anhydride in allethrin by the morpholine procedure (12) suffers from several disadvantages—the quantity of "acid chloride" must first be determined, the methanolic acid solution requires daily standardization, and, finally, a few allethrin samples have yielded negative anhydride values in the hands of the author. The latter anomaly is due to an interference with the color change at the end point, necessitating the addition of more than the theoretical quantity of reagent to bring the sample solution to match that of the blank. Substitution of a potentiometric titration would probably eliminate this effect.

The anhydride content of technical allethrin can be conveniently and rapidly determined at 5.56 microns (Figure 6). A standard curve, conforming to the Beer-Bouguer law, was drawn by employing samples of distilled allethrin to which had been added known amounts of purified chrysanthemummonocarboxylic acid anhydride. The concentration range examined was 0.1 to 6%. Attempts to determine the substance by the differential technique were unsuccessful, owing to poor instrument response even with wide slit widths and high amplification. Results obtained by the morpholine and infrared methods were in good agreement.

Allethrolone has been assayed by its ultraviolet absorption maximum at 227 m$\mu$ (5), but this method cannot be used in the presence of other alpha, beta-unsaturated carbonyl compounds. The keto alcohol can be determined by means of its 2.86-micron absorption band (Figure 7). Although satisfactory results have been obtained in the usual manner, by employing the differential technique the uncertainty accompanying the reading of absorbance values off the slope of a steep line is eliminated (Figure 8). Some preliminary work was carried out using the differential procedure in conjunction with an expanded wave-length scale (Figure 9), but the time consumed in calculating results outweighed the small increase in precision and accuracy. In actual practice transmittance paper is used and the area between 0% and sample amount of allethrolone is cut out and weighed analytically. All three techniques are accurate to ±0.02% in the concentration range 0.1 to 2.0%. The straight-line standard curves were plotted by measuring the absorbances of distilled allethrin containing varying amounts of purified allethrolone.

Although the author believes that the differential procedure is superior to the standard one, precautions must be taken to ensure that the allethrin used in the reference beam is completely free from allethrolone. Even when stored at 0° C. under nitrogen, purified allethrin develops very small amounts of allethrolone over a period of several months.

Alpha-dl-trans-allethrin (18) was first used to derive a standard curve at 5.81 microns. The analyses of several commercial allethrin samples based on this straight-line curve yielded results between 2.6 and 3.0% lower than the assays by the hydrogenolysis and ethylenediamine methods. Distilled allethrin (97.0% by both chemical procedures) was next employed as the standard. Good agreement was then observed among the three methods.

It was believed that the lack of concordance among the assays based on α-dl-trans-allethrin was due to a difference in absorb-
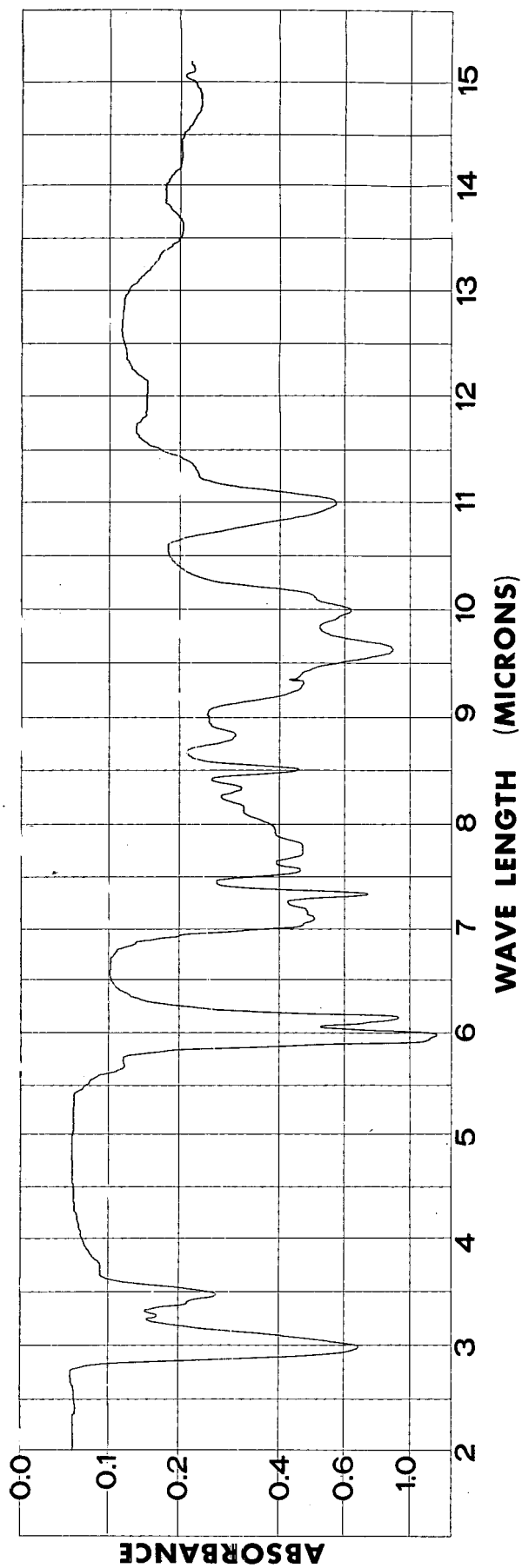
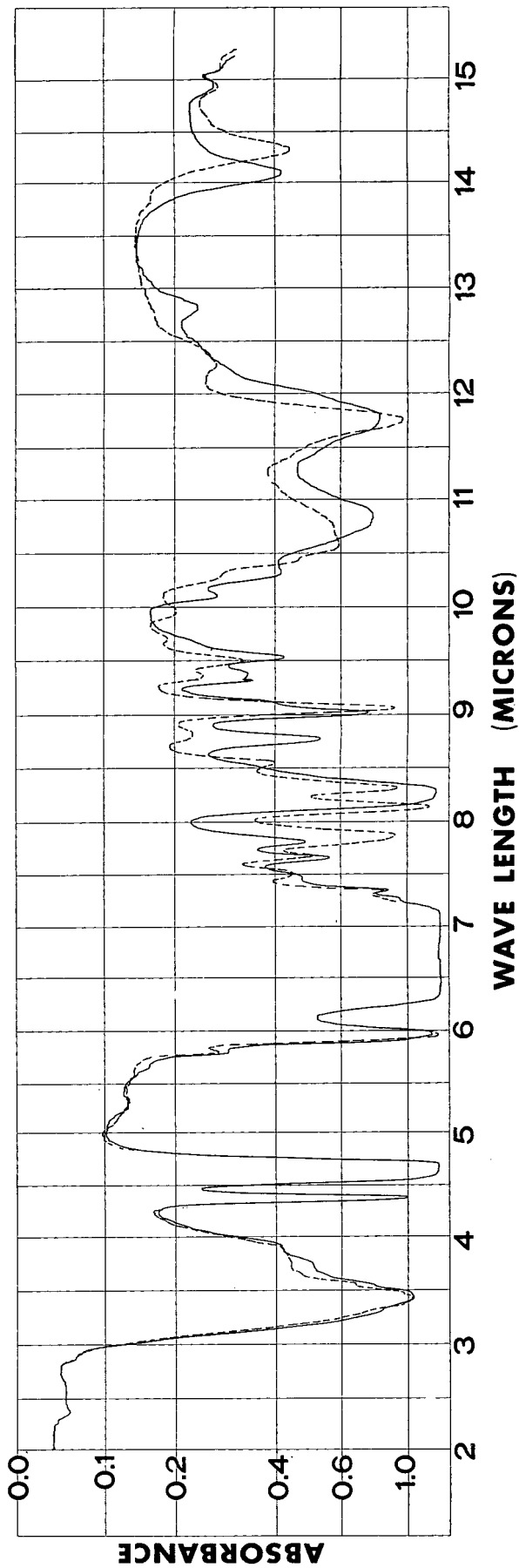Figure 2. Infrared spectrum of allethrolone (between salt plates)



Figure 3. Infrared spectra of chrysanthemummonocarboxylic acids

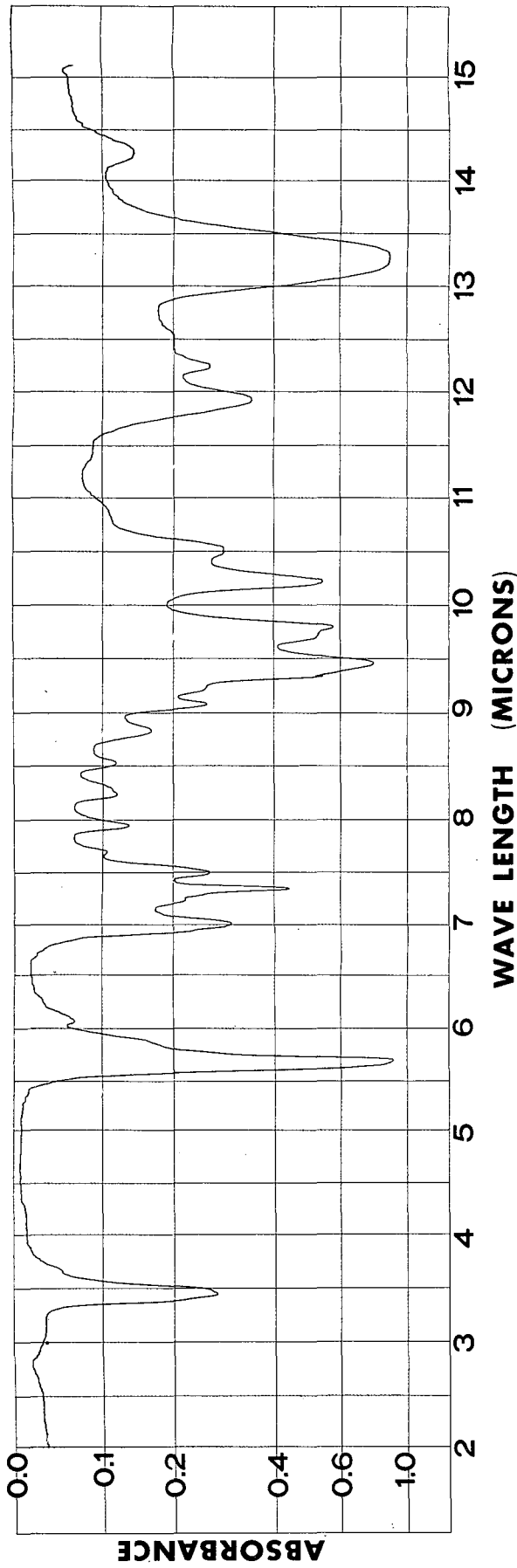——— cis, - - - trans, 2% in carbon disulfide, 0.5-mm. cell

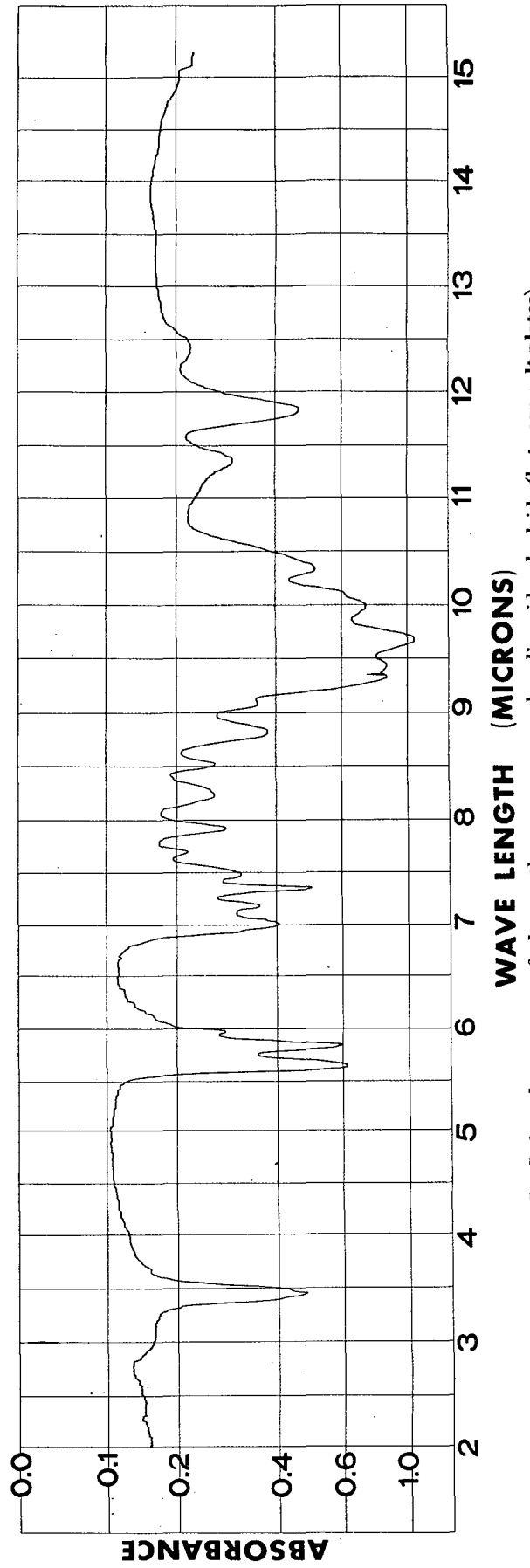Figure 4.   Infrared spectrum of chrysanthemummonocarboxylic acid chloride (between salt plates)

Figure 5.   Infrared spectrum of chrysanthemum-monocarboxylic acid anhydride (between salt plates)

ances of the cis and trans isomers. The two geometrical isomers of allethrin were prepared and their infrared spectra compared (Figure 10). The 8.7-micron (trans) and 8.85-micron (cis) bands were selected as the basis for an analytical procedure and a straight line was obtained when log ratio of absorbances was plotted against per cent *trans*-allethrin content.

The commercial allethrin samples studied displayed a fairly constant cis-trans ratio (20 to 80). Owing to the fact that the infrared assay shows *cis*-allethrin to be 14% lower than the trans isomer, a correction factor of 0.14% must be applied for each per cent variation from 80.0% trans, the amount present in the distilled allethrin standard.

Apparently little change in cis-trans proportion occurs in the preparation of allethrin according to the synthesis of Schechter,

**Figure 6.  Anhydride content of technical allethrin**

Green, and LaForge (17). The first intermediate exhibiting geometrical isomerism is ethyl chrysanthemumate, and the acid was isolated by saponifying the ester with 0.5N alcoholic potassium hydroxide. Pure *cis*- and *trans*-chrysanthemummonocarboxylic acids were prepared and their spectra recorded (Figure 3). The absorption bands at 14.04 (cis) and 14.26 (trans) were selected, and a standard curve was drawn. The acid derived from the ester assayed 77% trans.

### DISCUSSION OF RESULTS

More than 50 commercial allethrin samples were analyzed by the infrared method described in this paper and checked by the hydrogenolysis and ethylenediamine procedures. Twelve representative analyses are outlined in Table I.

The results of both the ethylenediamine and infrared analytical methods are in excellent agreement in all instances except for sample E, where there is a difference of 0.8%. With the exception of samples G and H, the hydrogenolysis assays compare favorably with those of the other two methods. However, the author has not found the hydrogenolysis procedure completely reliable when applied to some commercial allethrin samples less than 90% pure. The source of the insecticide appears to be important, for erratic behavior has been observed in some samples (G and H, Table I).

Once the standard curves have been drawn, determination of allethrin requires less time by infrared spectrophotometry than by the chemical methods. Gersdorf and Mitlin (7) report that *dl-trans*-allethrin is approximately 50% more active than *dl-cis*-allethrin.

Preparation of ethyl chrysanthemumate by methods other than that described by Schechter, Green, and LaForge could conceivably yield an insecticide with a different cis-trans ratio. None of the chemical methods for determining the purity of allethrin is able to detect an important alteration of the molecule of this type, which directly affects its entomological activity.
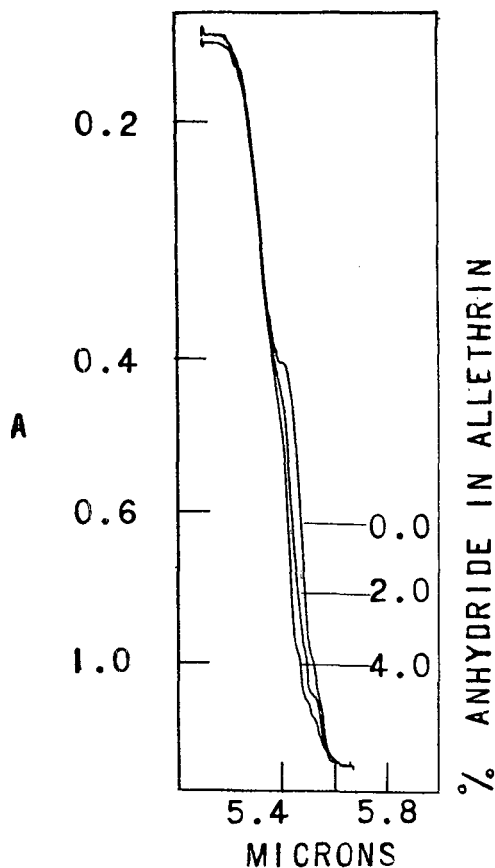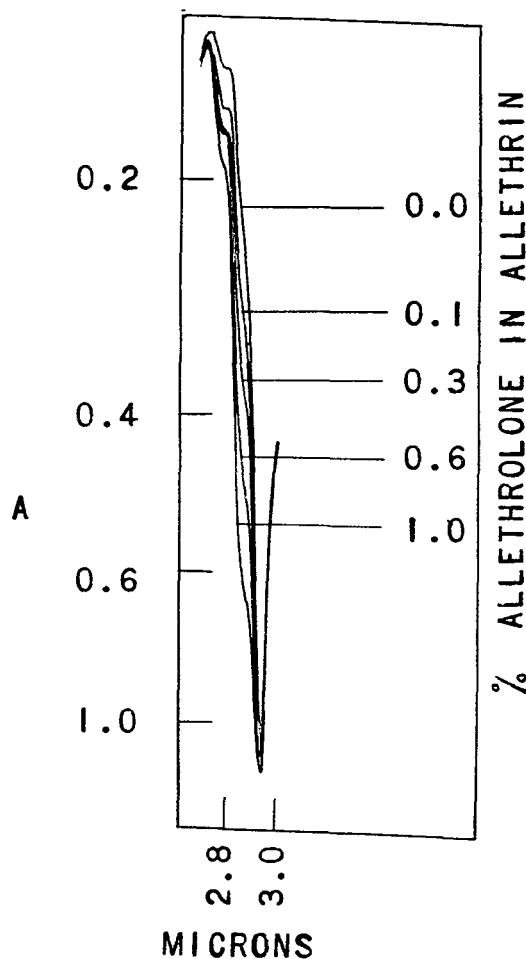
**Figure 7.  Determination of allethrolone in allethrin by standard technique**

The data of Gersdorf and Mitlin (7) indicate that allethrin consists of 69% cis and 31% trans isomers, in direct contradiction to the present paper's findings. In the light of the numerous analy-

ses conducted in the author's laboratory, the entomological findings should be viewed with suspicion.

### EXPERIMENTAL

A Perkin-Elmer No. 21 double-beam recording spectrophotometer was employed with the controls set for quantitative operation as recommended by the manufacturer. Standard curves were plotted as absorbance (A) vs. concentration. Where solutions were used, at every analytical wave length the absorbance due to the solvent alone was subtracted from that of the sample plus solvent.

**Allethrolone** (dl-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one). The keto alcohol was prepared by the method of Schechter (17) and purified through its semicarbazone (5). The material assayed 99.5% by the ultraviolet absorption method (5). Three

percentage of allethrolone is read off the standard curve. Between 0.1 and 2.5% allethrolone can be determined by this procedure. Larger amounts can be analyzed by decreasing the cell thickness.

B. DIFFERENTIAL METHOD. A 0.5-mm. cell containing purified allethrolone is placed in the reference beam and the material under test (0.5-mm. cell) is put in the path of the sample beam. The spectrum is scanned between 2 and 3 microns. The absorbance obtained by placing purified allethrolone in both cells is subtracted from the total absorbance found at 2.86 microns and this corrected value is used in reading the quantity of allethrolone present from the standard curve. Between 0.05 and 2.5% can be determined employing this technique.

C. DIFFERENTIAL METHOD WITH EXPANDED SCALE. Two 0.5-mm. sealed cells were used as in Method B. The instrument gears were changed to increase the wave-length scale to 160 cm. per micron and the spectrum was recorded from 2 to 2.9 microns.
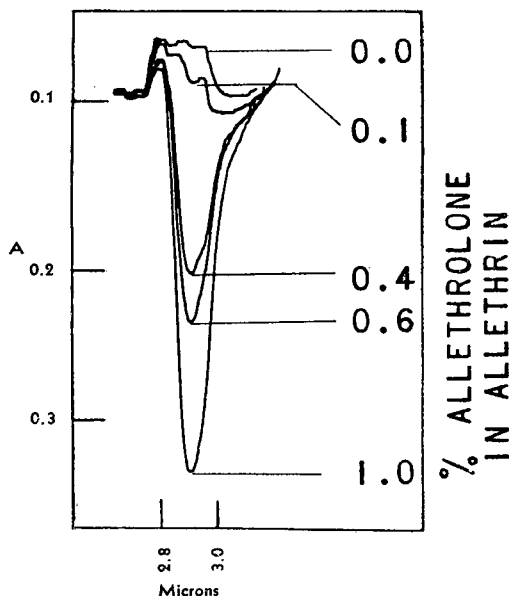
**Figure 8. Determination of allethrolone in allethrin by differential technique**

**Table I.  Infrared and Chemical Determination of Allethrin**

| Sample | Allethrolone | Anhydride | Trans | I.R. | H₂ | E.D. |
|---|---|---|---|---|---|---|
| | | | | | Purity | |
| A | 0.10 | 0.79 | 80.0 | 93.9 | 93.6 | 93.8 |
| B | 0.20 | 1.9 | 80.5 | 92.3 | 92.1 | 92.0 |
| C | 0.15 | 2.1 | 80.2 | 92.8 | 93.1 | 93.0 |
| D | 0.11 | 0.80 | 80.9 | 93.6 | 93.9 | 93.6 |
| E | 0.10 | 0.00ᵃ | 80.1 | 93.9 | 94.6 | 94.7 |
| F | 0.21 | 1.5 | 80.5 | 93.0 | 94.0 | 93.1 |
| G | 0.07 | 0.42 | 81.0 | 90.4 | 87.0 | 90.5 |
| H | 0.13 | 0.65 | 79.5 | 90.6 | 87.5 | 90.5 |
| I | 0.22 | 3.1 | 80.4 | 94.5 | 94.6 | 94.2 |
| J | 0.10 | 0.10 | 80.4 | 94.7 | 94.3 | 94.5 |
| K | 1.00 | 0.40 | 80.0 | 95.3 | 95.5 | 95.2 |
| L | 0.25 | 2.5 | 80.2 | 94.8 | 94.9 | 94.5 |

ᵃ Morpholine method yielded (−)1.0%.

A blank was run with purified allethrolone in both cells and the area between the blank and sample curves was cut out and weighed on an analytical balance. The allethrolone content was then read from a standard curve which was plotted in weight of paper vs. percentage of allethrolone. Between 0.03 and 4% can be determined by this technique.

**Chrysanthemummonocarboxylic Acid Anhydride.** The anhydride was prepared for the first time by reaction of equimolar quantities of the acid chloride and sodium chrysanthemumate in a flask under nitrogen. When the liberation of heat ceased, the vessel and contents were heated on a steam bath for 1 hour.
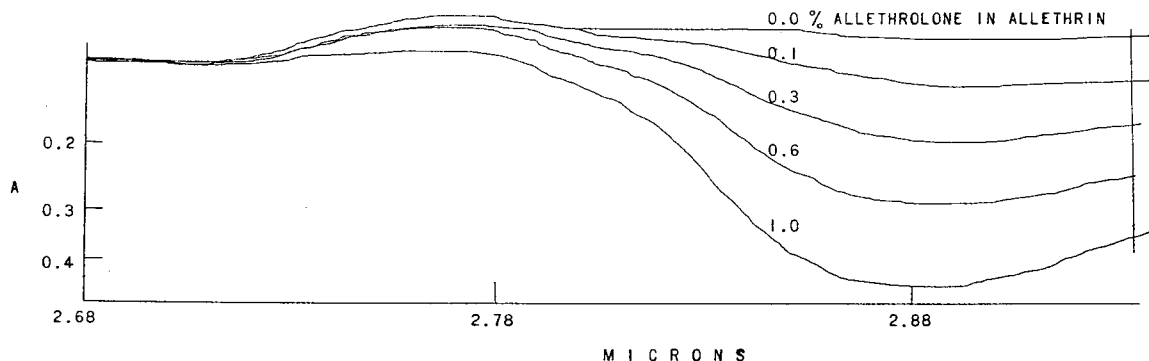
**Figure 9.  Determination of allethrolone by differential technique with expanded wave-length scale**

different techniques were employed in determining small amounts of allethrolone in allethrin.

A. STANDARD PROCEDURE. A 0.2-mm. sodium chloride sealed cell is filled and the spectrum recorded from 2 to 3 microns. The absorbance at the 2.86-micron maximum is noted and the

The mixture was then extracted with ether and the ether was dried and distilled off. On distilling at 188° to 189° C. and 10 mm., a pale yellow, odorless liquid was obtained in 55% yield assaying 98.5 to 99.0% anhydride by the morpholine procedure (12). The anhydride contained 2% chrysanthemummonocarboxylic acid as determined by potentiometric titration to pH 9.5 in 50%
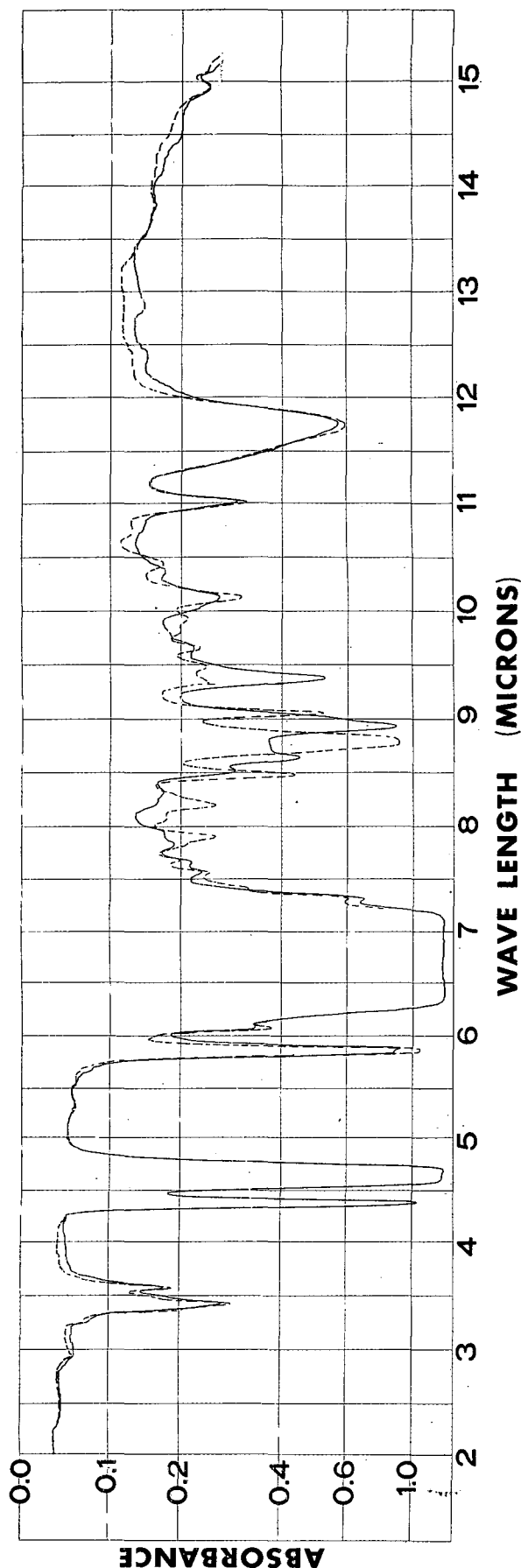
Figure 10. Infrared spectra of allethrin

—— cis-allethrin, - - - trans-allethrin, 2% in carbon disulfide, 0.5-mm. cell

WAVE LENGTH (MICRONS)

ABSORBANCE

ethyl alcohol–water solution. The anhydride was also prepared by refluxing chrysanthemummonocarboxylic acid with a large excess of acetic anhydride, but a lower yield of material assaying 88% resulted.

For the determination of anhydride in allethrin a 0.1-mm. cell was employed and the spectral region between 4 and 5.8 microns recorded. The analytical wave length selected was 5.56 microns; anhydride in the range of 0.1 to 6% can be determined.

**Allethrin.** The insecticide was prepared as directed in the literature (17) and approximately 500 grams were fractionated at 1 mm. A middle cut was taken and, when assayed by both hydrogenolysis and ethylenediamine methods, was found to contain 97.0% allethrin. Beer-Bouguer's law was followed in the range 0.18 to 0.28 gram per 100 ml. of carbon tetrachloride at 5.81 microns. More than 50 points were taken over a period of 2 months for the preparation of the standard curve.

For the analysis, 0.2 to 0.25 gram of allethrin was dissolved in 100 ml. of carbon tetrachloride, and transferred to a 0.5-mm. sealed absorption cell, and the region between 5.2 and 6.0 was recorded. The percentage of allethrin was then read from the standard curve. A correction must be made for the allethrolone present in the sample, by subtracting the allethrolone content multiplied by a factor of 1.1 from the total allethrin found. When anhydride is present in more than 5% amounts, an additional correction must be made. However, no commercial samples examined contained amounts necessitating a correction. Finally, the percentage of trans-allethrin present must also be determined and a correction made for it.

**cis- and trans-Allethrin.** The two geometrical isomers were prepared according to the method of Schechter (17). Prepared mixtures of the isomers were dissolved in carbon disulfide (2 grams per 100 ml.) and the spectra run in a range 8.4 to 9 microns in a 0.2-mm. cell. Log ratios of absorbances at 8.7 microns (trans) and 8.85 microns (cis) were plotted against percentage trans isomer to yield a straight-line standard curve. Straight-line relationships between log A and concentration indicate to the reader that, although the solution does not follow Beer's law, the shape of the standard curve is hyperbolic in nature. A correction factor of 0.14% must be applied for each percentage variation from the trans content of the standard allethrin (80.0%).

**cis- and trans-Chrysanthemummonocarboxylic Acids (9, 18).** One gram of acid is dissolved in 100 ml. of carbon disulfide and transferred to a 0.5-mm. cell. The spectral region recorded lies between 13.6 and 14.6 microns. The cis form exhibits a maximum at 14.04 microns and the trans form at 14.26 microns.

Chrysanthemummonocarboxylic acid ethyl ester used in the synthesis of the acids studied was prepared by the addition of ethyl diazoacetate to 2,5-dimethyl-2,4-hexadiene (2, 17).

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# Coulometric Determination of Ferrocyanide with Electrolytically Generated Ceric Ion

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Samples of potassium ferrocyanide from 1.7 to 62 microequivalents have been titrated with electrolytically generated ceric ion with an accuracy of ±0.40% or better. The sensitive amperometric end point was employed. The chemical reaction near the equivalence point was slow, but by generating in small increments in this region, and waiting a few minutes for equilibrium to become established, accurate results were obtained. Very dilute potassium ferrocyanide solutions were found to be unstable so that it was necessary to use, as samples, weighed aliquots of 0.01N solutions, which determined the lower limit of samples that could be measured accurately.

THE determination of ferrocyanide both for its own inherent value, and as a reduction product in the indirect determination of other substances—e.g., hydrazine, reducing sugars, etc.—has become increasingly important. Ceric sulfate has been used as a volumetric oxidizing agent in this determination by numerous investigators. The end point of this determination has been detected by visual indicators such as 1,10-phenanthroline ferrous sulfate (10), diphenylamine sodium sulfate (2), or the greenish color of ferric ferrocyanide (6), and potentiometric methods (1, 6, 9). Of the workers who have studied this reaction, Berry (2) gives few data and Willard and Young(10) merely tabulate general conditions for the titration. Furman and Evans (6) have made a detailed study of the conditions that are optimum for the direct determination of ferrocyanide, and also for the titration of ceric ion with ferrocyanide. Most of the conditions for the titrations performed in this investigation were based on the material presented by these last authors.

Relatively few inorganic substances have been determined coulometrically using electrolytically generated ceric ion. To date those include ferrous iron (5), uranium (4), and iodide ion (7) but not ferrocyanide. Since a sensitive method for determining microquantities of ferrocyanide might be developed by means of a coulometric procedure, the present study was directed toward this aim.

## APPARATUS

Figure 1 shows a schematic representation of the apparatus used for the coulometric titrations. The constant current source, designed by Reilley, Cooke, and Furman (8), is capable of delivering currents from 1 to 5 ma. and from 25 to 150 ma., in multiples of 25 ma. To obtain current values intermediate to these, several carbon resistors were included in the external circuit. When working with currents between 25 $\mu$a. and 1 ma., the apparatus was used as a constant voltage source with high resistors, which could be switched in series with the voltage supply to provide the desired current intensity (see Figure 1). Two Ohmite, Type AB, variable carbon resistors were included in the circuit in order to make fine adjustments of the current value. When large currents of 10 ma. or larger were used, these potentiometers were short-circuited to prevent them from being burned out. A 10-ohm wire-wound resistor was included in the circuit as a dummy for the titration cell. Thus, very little change occurred in the resistance in the circuit when the current was permitted to flow through the titration cell.

The generating current was measured by determining the $IR$ drop across a standard 10,000-ohm resistance box with a Leeds

and Northrup student-type potentiometer. All time measurements were made with a Model S-6, clutch-operated clock from the Standard Electric Time Co.

The sensitive end-point procedure of Cooke, Reilley, and Furman (3) was used for detection of the end of the titration. The circuit for this consisted of a Leeds and Northrup Type P galvanometer, a Shallcross Aryton shunt, and two 1.5-volt dry cells, which served as a voltage source.

The generator electrodes were of platinum-iridium foil. The anode was 2 × 4 cm. and the cathode was 1 × 2 cm. The cathode was isolated from the bulk of the solution in the titration vessel by a separate compartment, which had a sintered-glass disk at its bottom and was filled above the level of the solution in the titration cell with 15% ammonium sulfate solution. Potassium sulfate and sodium sulfate could not be used in this compartment when working with the cerous sulfate generating solution for a precipitate of cerous alum was formed from the small amount of salt that leaked from the cathode compartment into the titration vessel. The presence of this precipitate hindered the proper functioning of both the generator anode and the indicator electrodes.

The operating-indicator electrode consisted of a 2-cm. long platinum wire, B and S gage 28, for the larger samples, but with microsamples, where a greater sensitivity was required, a 1-cm. square platinum-iridium foil was substituted for the wire. The reference cell consisted of a saturated cell of lead amalgam–lead sulfate in 1N sulfuric acid, as recommended by Cooke,· Reilley, and Furman (3). This cell has a constant potential of −0.27 volt versus the hydrogen half-cell and has the advantage that it introduces no interfering ions into the titration cell.

A No. 7664 Leeds and Northrup pH meter was used to present the potential of the indicator circuit for the sensitive end-point procedure.

The titration cell consisted of a weighing bottle of a 30-ml. capacity, covered with a rubber stopper provided with openings for the four electrodes, a gas inlet, and the addition of the sample.

All samples were added to the titration cell from a 5.000-ml. microburet graduated in 0.01 ml., except those of 1.8-microequivalent size, which were added from a BD hypodermic syringe of 1.0-ml. capacity, that served as a weight buret. A piece of glass tubing was drawn out to provide a fine tip for delivery of the sample.

## SOLUTIONS

The generating solution was prepared by dissolving reagent grade cerous sulfate tetrahydrate (G. F. Smith Chemical Co.) in 2.0N sulfuric acid until the solution was saturated (ca. 12%).

The ferrocyanide solutions were prepared by dissolving weighed amounts of reagent grade potassium ferrocyanide trihydrate (Baker and Adamson) in 2.0N sulfuric acid which had been deaerated with tank nitrogen, and by making up to volume with deaerated water. These solutions were standardized by titration with 0.05205N ceric sulfate, standardized against arsenious oxide, using 1,10-phenanthroline ferrous sulfate as an indicator. The ferrocyanide solutions were found to be somewhat unstable, making it necessary to prepare and standardize a fresh solution each day. When not in use throughout the course of the day, they were kept in the dark in order to minimize decomposition.

## PROCEDURE

Eighteen milliliters of the saturated cerous sulfate solution were placed in the titration cell and deaerated with tank nitrogen for 15 minutes. For samples of 1.8 microequivalents, 3.0 ml. of sirupy (85%) phosphoric acid also were added to eliminate a drift in the indicator system. After deaeration, the potential of this solution was adjusted to 1.210 volts as indicated by zero current flow on the galvanometer. Since the potential of the generating solution was usually below this value, this presetting of the solution potential was easily accomplished by merely generating ceric ion. However, occasionally it was found necessary to add a drop of ferrocyanide to reduce the potential to a value below the reference one, and then generate ceric ion. Then

the sample of ferrocyanide was added to the cell and generation of ceric ion at constant current begun and continued until zero current reading on the galvanometer was obtained again. In the vicinity of the end point, where the concentration of the reacting ions was very low, generation was carried out in small increments with a wait of 5 minutes (10 minutes in the case of a second or third sample titrated in the same background mixture) to allow equilibrium to be established. The number of microequivalents of ferrocyanide were calculated from the time of generation and the value of the current. The results of a series of such determinations are presented in Table I.

### DISCUSSION

The potential impressed across the indicator electrodes (1.210 volts) was determined by potentiometric titration of ferrocyanide with electrolytically generated ceric ion. The potential of the midpoint of the break in this titration curve was selected as the most satisfactory one to use as the preset end-point potential.

**Table I.   Coulometric Determination of Ferrocyanide with Electrolytically Generated Ceric Ion**

| Current, Ma. | Time, Min. | Microequivalents | | | Error, % |
|---|---|---|---|---|---|
| | | Added | Found | Difference | |
| 11.91 | 8.419 | 62.29 | 62.34 | +0.05 | +0.08 |
| 11.92 | 8.393 | 61.93 | 62.13 | +0.20 | +0.32 |
| 11.90 | 8.290 | 61.68 | 61.34 | −0.34 | −0.55 |
| 12.05 | 8.223 | 61.62 | 61.61 | −0.01 | −0.02 |
| 4.090 | 12.139 | 30.94 | 30.87 | −0.07 | −0.23 |
| 4.090 | 12.024 | 30.69 | 30.58 | −0.11 | −0.36 |
| 4.130 | 11.919 | 30.69 | 30.61 | −0.08 | −0.26 |
| 4.100 | 12.003 | 30.63 | 30.60 | −0.03 | −0.10 |
| 1.087 | 8.135 | 5.492 | 5.498 | +0.006 | +0.11 |
| 1.090 | 8.039 | 5.449 | 5.448 | −0.001 | −0.02 |
| 1.090 | 8.027 | 5.435 | 5.440 | +0.005 | +0.09 |
| 1.090 | 7.653 | 5.179 | 5.187 | +0.008 | +0.15 |
| 0.4962 | 6.304 | 1.949 | 1.945 | −0.004 | −0.21 |
| 0.4929 | 6.071 | 1.867 | 1.861 | −0.006 | −0.32 |
| 0.4956 | 5.837 | 1.794 | 1.799 | +0.005 | +0.28 |
| 0.4942 | 5.634 | 1.734 | 1.731 | −0.003 | −0.23 |

Deaeration of the cerous sulfate generating solution was necessary to obtain precise results, for without it the solution became pale blue upon addition of the sample and the results were from 4 to 13% too low. It was found necessary to disconnect the indicator electrodes throughout the major part of the titration and only have the detection circuit in operation during the last minute of the titration. Otherwise a slight blue precipitate formed on the platinum indicator electrode, which caused the results to be about 1% too low. The indicator electrode was cleaned readily by placing it in boiling 1 to 1 nitric acid for 10 minutes and then holding it in a soft flame for a few minutes.

If the generation of ceric ions was carried out continuously until the galvanometer reading was zero, the results were again low. When the generation was halted there was no noticeable drift in the downward direction of potential readings at this point; thus the reaction appeared to have gone to completion. However, if the generation was stopped before the galvanometer reached a reading of zero current, a slight downward drift was noted, indicating a sluggishness in the reaction near the equivalence point. This is not entirely unexpected from a consideration of the sizes and concentrations of the ions involved at this portion of the titration curve. Furman and Evans (6) also report this in their macrotitrations and state that from 1 to 3 minutes are required in the immediate vicinity of the end point to obtain stable potential readings. In this work, 5 to 10 minutes were found to be necessary for the attainment of stable galvanometer readings—i.e., two readings on the galvanometer scale were identical for 1 minute. When a second sample had been added to the same solution in the cell after completion of one titration the longer period of time was necessary, while for the first sample in a

background medium, a wait of 5 minutes was found to be generally an adequate period.

In general, the ferrocyanide solutions were sufficiently stable throughout an 8- or 9-hour period to permit their use within that length of time without restandardization. However, a decrease, observed in the titer in the case of the 0.003N solution, was so slight that no significant error was introduced through its continued use. In the case of 0.001N ferrocyanide, the decay in titer was so rapid that successive titrations, when standardizing the solutions, gave significantly lower values (approximately 0.20 ml. of the ceric sulfate) each time. Also, within 2 hours the solution had decreased in concentration from 0.001103 to 0.001032N. Thus, it was impossible to use solutions that were this dilute. Therefore for the 1.8-microequivalent samples, 0.01N ferrocyanide was used, and the hypodermic syringe was employed for the addition of the sample instead of the microburet. With ferrocyanide solutions of this concentration, samples much smaller than 1.8 microequivalents could not be determined conveniently with great precision since aliquots would have had weights only slightly greater than 100 mg. Perhaps the use of 0.005N ferrocyanide solutions would permit the determination of 1 microequivalent (422 γ of ferrocyanide), but with samples smaller than that, the accuracy of the measurement of sample size would be less than that of the determination of the concentration itself.
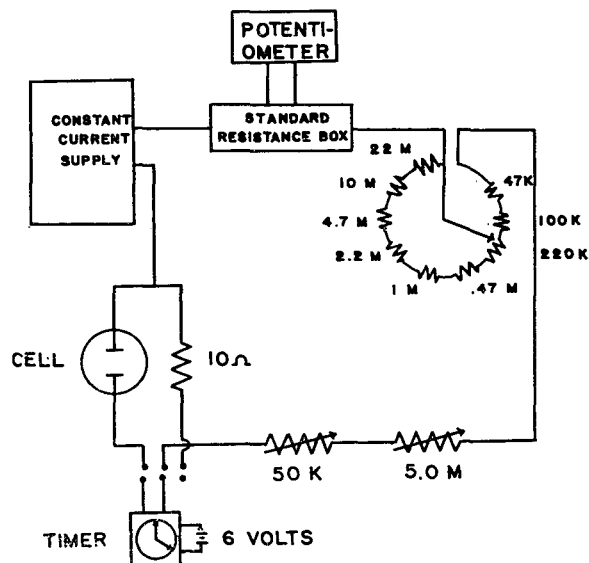


**Figure 1.   Schematic generation circuit**

The data in Table I show that samples of ferrocyanide from 1.7 to 62 microequivalents (or 0.72 to 26.2 mg.) can be determined with an accuracy of ±0.40 %, by a coulometric procedure. By using higher generation currents, larger amounts certainly could be determined and perhaps smaller samples could be titrated, although the accuracy of the knowledge of the amount of sample added becomes less as the sample size decreases.

It should also be possible to apply this determination of ferrocyanide to such procedures as those involving reducing sugars and other substances in which the ferrocyanide produced by the reduction of ferricyanide is a measure of the amount of material present.

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# Thermogravimetric Pyrolysis of Cupferron Complexes of Scandium, Yttrium, and Rare Earth Elements

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The thermogravimetric pyrolysis of the scandium,
yttrium, and the rare earth cupferrates was determined.
It was found that it is not necessary to ignite the com-
plexes to 900° C. but that the oxide level is reached at
500° to 600° C. The construction and operation of a
simple thermobalance are described.

THE use of cupferron (ammonium salt of N-nitroso-phenyl-
hydroxylamine) as a precipitant for the rare earth elements
has recently been investigated (5). It was found that the rare
earth elements—lanthanum, cerium(III), praseodymium, ne-
odymium, samarium, and gadolinium—were quantitatively pre-
cipitated from solution at a pH of 3 to 4. As the precipitates
were contaminated with an excess of cupferron, they were ig-
nited and weighed as the corresponding oxides. This study was
undertaken to determine the optimum temperature limits for the
ignition to the oxide and also to investigate any intermediate
products that may be formed during the decomposition.

## EXPERIMENTAL

**Reagents.** Cupferron was obtained from the G. F. Smith
Chemical Co., Columbus, Ohio, and the Matheson, Coleman,
Bell Co., East Rutherford, N. J. It was used without further
purification.

The rare earths were obtained as the oxides of better than 99%
purity from Research Chemicals, Inc., Burbank, Calif., and the
Lindsay Chemical Co., West Chicago, Ill. As the contaminants
were other rare earth elements, no further purification was neces-
sary.

Scandium oxide of 99.8% purity was obtained from A. D.
Mackay, Inc., New York, N. Y.

Yttrium chloride of 99% purity was obtained from Research
Chemicals, Inc., Burbank, Calif.

All other reagents were of analytical reagent grade.

**Thermobalance.** In a recent book, Duval (1) summarizes the
development of the thermobalance. He found that if a standard
beam balance were used, the vibration soon dulled the knife edges
and could also result in the beam's being displaced from the center
position. These difficulties were overcome by the use of a
multiple-range, precision torque balance. The balance was con-
verted into a thermobalance as shown in Figure 1. The torque
balance, 0 to 100 mg. in range, was made by the Vereenigde
Draadfabrieken, Nijmegen, Holland. The smallest scale division
was 0.2 mg.; thus, weighings could be made to ±0.1 mg. The
sample was placed in a platinum boat, 1 cm. in diameter, sus-
pended by a platinum wire, in a Vycor glass tube, 2.5 cm. in
diameter and about 25 cm. in length. This tube was connected
to the balance by a $ 29/42 joint at B. The furnace, A, was
constructed by first winding 15 feet of No. 22 gage, Nichrome
alloy V, resistance wire (1.01 ohms per foot), into a coil ⅛ inch
in diameter, then winding this coil onto the asbestos-covered
tube at about ¼-inch spacings. The completed windings were
covered adequately with asbestos insulation.

The heating rate of the furnace was controlled by a 6 revolu-
tions-per-day 110-volt synchronous motor connected to the shaft
of a Powerstat. The heating rate was linear from 35° to 950° C.,
at about 4.5° per minute. A slower heating rate could be ob-
tained by decreasing the input voltage into the motor-driven
Powerstat.

The temperature of the furnace was measured by an iron-
constantan thermocouple, T, using an ice bath as the reference
junction. The potential of the thermocouple was detected by a
Gray portable potentiometer, Model E-3042-S, made by the Gray
Instrument Co., Philadelphia, Pa. The thermocouple was
calibrated against the freezing point and boiling point of water,
and the freezing points of cadmium metal and potassium chloride.
The electromotive force of the thermocouple could be read to
within ±0.05 mv., which corresponds to about ±1°.
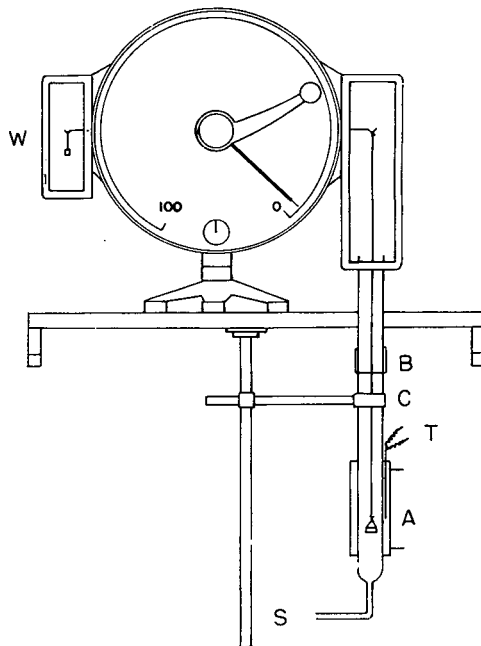


**Figure 1. Schematic diagram of thermo-
balance**

It was found that during the pyrolysis of the cupferron com-
plexes, the decomposition products adhered to the sides of the
tube and the suspending wire. To prevent this difficulty, a suc-
tion was applied to the tube, S, which allowed a slow stream of air
to pass over the sample. No adverse effects could be detected

by the balance. Usually, a bubble count of 3 per second was used.

The accuracy and reproducibility of the thermobalance compared favorably with the Chevenard recording balance (1). This was shown by the thermal decomposition of $Na_2HPO_4.12H_2O$. The resultant curve agreed to within 1% of that previously determined.

**Procedure.** The cupferron complexes of scandium, yttrium, and the rare earth elements were prepared as previously described (5). The complexes with scandium and yttrium have not been previously reported, although Pokras (4) noted that cupferron precipitated scandium quantitatively from a neutral solution. Coprecipitation was observed as with the rare earth complexes as the ratios of metal to cupferron found were slightly greater than 1 to 3. All of the precipitates were dried at room temperature for at least 24 hours before being pyrolyzed on the thermobalance.

The decomposition of the complexes was carried out by placing 100 to 200 mg. of the dried complex in the platinum boat and suspending it in the furnace. Since the torque balance has a range of 0 to 100 mg., a counterweight was attached at W. The motor-driven Powerstat was set at 0 volts, and the input voltage was adjusted to 60 volts by means of another Powerstat. After the rate of air flow through the furnace was adjusted, the weight of the sample in the boat was determined, and the synchronous motor was started. Readings on the balance and the potentiometer were taken at short intervals until a temperature of about 800° C. was reached. Each decomposition took about 3.5 hours to complete. At least two runs were made of each complex with agreement to within 1%.

### DISCUSSION

The pyrolysis curves are shown in Figures 2 and 3. The curves of lanthanum, praseodymium, neodymium, samarium, and scandium are similar in appearance. The thermal stabilities of these rare earth cupferrates are remarkably higher than those of any other cupferrates so far determined. Previously reported iron-(III) and copper(II) cupferrates were stable to 98° and 118° C., respectively (3). In the case of the rare earth cupferrates, the first decomposition begins at about 150° to 180° C. where there
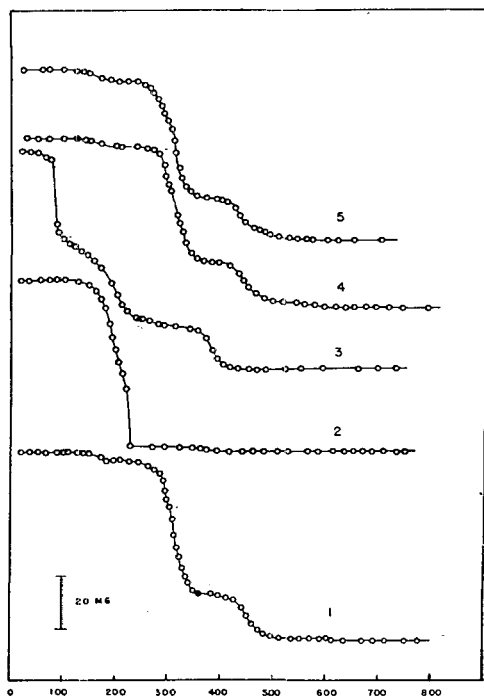
**Figure 2. Pyrolysis curves of rare earth cupferrates**

1. Lanthanum cupferrate
2. Cerium(III) cupferrate
3. Cerium(IV) cupferrate
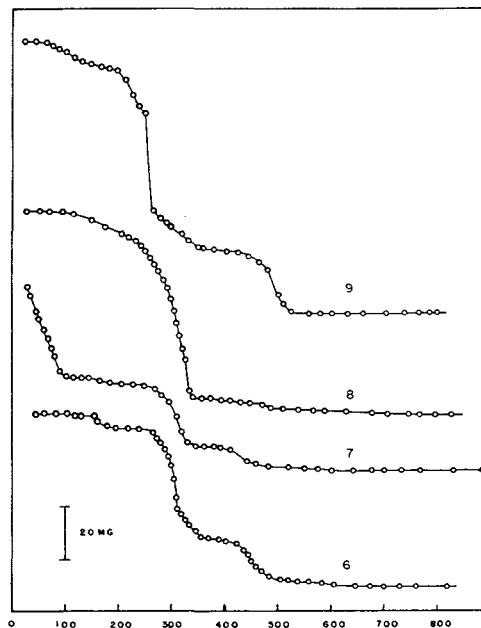4. Praseodymium cupferrate
5. Neodymium cupferrate

**Figure 3. Pyrolysis curves of scandium, yttrium, and rare earth cupferrates**

6. Samarium cupferrate
7. Gadolinium cupferrate
8. Yttrium cupferrate
9. Scandium cupferrate

is a small break in the curve and then a plateau extending to about 280° to 290° C. This break has also been observed in the case of aluminum cupferrate (2) but at a corresponding lower temperature. Another decomposition then occurs and a slight plateau is again formed, which decomposes to the oxide at 450° to 600° C. As far as could be determined, the intermediate plateaus do not correspond to any stoichiometric compositions and are probably mixtures. The melting points of the rare earth cupferrates are from 182° to 188° C., very close to the point where the first decomposition occurs.

The pyrolysis curves for cerium(III) and yttrium are similar. No intermediate plateaus were observed, the decomposition proceeding directly to the corresponding oxides.

The least stable of the complexes seem to be those of cerium(IV) and gadolinium. When anhydrous, they begin to decompose at room temperature. This would probably be expected of cerium(IV) because of its tendency to revert to the lower oxidation state, but the instability of the gadolinium complex cannot be explained.

### CONCLUSION

It hardly seems necessary to ignite the cupferron complexes to 900° C. as previously described. This temperature is far too high, as the oxide level is reached at 500° to 600° C. The intermediate products formed in the case of the lanthanum, praseodymium, neodymium, samarium, gadolinium, and scandium complexes are being further investigated.

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# Rapid Separation of Tracer Amounts of Rare Earth Elements of the Yttrium Group

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A technique having possible applications in neutron activation analysis separates tracer amounts of the heavy rare earth elements in 30 minutes or less. The method is based on the use of buffered glycolic acid solution as an eluting agent for selectively removing the rare earths from cation exchange resin columns 1 cm. or less in height. Data are presented relative to the importance of controlling the resin size, its initial chemical form, and the elutrient flow rate. Commercial glycolic acid contains calcium ion, which must be removed from the elutrient solution used with these very short resin columns.

I N RADIOCHEMISTRY, or in neutron activation analysis, there is often a high premium on speed in separation methods because of the short decay periods of the desired radioactive products of an irradiation. Where these short-lived species are nuclides of the rare earth elements, attainment of such rapid separations has been particularly difficult, save for the few members of the series having readily accessible oxidation states other than the characteristic one of plus three. Some techniques are described below whereby tracer quantities of the heavier rare earths can be separated to a useful degree in a matter of minutes by use of ultrashort cation exchange resin columns with a buffered glycolic acid eluting agent.

Glycolic (hydroxyacetic) acid was one of the materials evaluated by Mayer and Freiling (2) as a possible eluting agent for separating rare earth ions on resin columns. For this particular acid, only a single set of data was presented, but this was so promising that Studier, Mech, and Gindler (5) of the Argonne National Laboratory nuclear chemistry group have studied the system further and have adapted it for use in separating the rare earthlike plus three ions of the transplutonium elements. Its marked success in this application made further detailed study of its use for rare earth element separations seem desirable; the study has been undertaken and is still in progress.

The data presented by Mayer and Freiling were obtained from a resin column operated at 87° C. Satisfactory separations can also be attained at room temperature if the pH of the buffered eluting solution is lowered. If this process of lowering the pH is carried relatively to an extreme, it will be possible to obtain usable separations with resin columns 1 cm. or less in height. If a resin of very small particle size is used, high flow rates are also feasible at the same time. The combination of a short column and high flow rate leads to very rapid separations of the members of the yttrium group of the rare earth series, as they are the first to come off the column.

The nature of the cation in combination with the resin initially will have a marked effect on the degree of separation, and particular care must be taken to avoid having any of the resin in the calcium form at the start.

## MATERIALS, EQUIPMENT, AND PROCEDURE

**Resin.** Ten pounds of −400-mesh, 12% cross-linked Dowex 50 were suspended in 2 feet of distilled water in a large battery jar. Four fractions were taken as the material settled: material settling in less than 10 minutes, 10 to 30 minutes, 30 minutes to 2 hours, and 2 to 18 hours. This last, finest fraction was used for all the tests described, save where the resin size effect itself

was under study. Before use, all resin batches were treated several times with concentrated hydrochloric acid and with ammonium citrate solutions to remove as much iron as possible (a common contaminant of commercial resins). After the columns had been filled, and between runs where the same column was re-used, a number of column volumes of 0.25M ammonium citrate were passed in order to remove any residual radioactivity and to ensure that the resin would be in the ammonium form. The columns were then washed with water and filled with eluting agent before use.

**Glycolic Acid Solutions.** Matheson, Coleman, and Bell crystalline glycolic acid was employed as a 0.25M aqueous solution. This solution was passed through a bed of Dowex 50 resin in the hydrogen form to remove contaminating cations (particularly calcium). In some cases a wetting agent (Aerosol OT) was added to the glycolic acid solution at this point to produce a concentration of 0.05%. The pH was then adjusted to the desired level with ammonia gas.

**Radioactive Tracers.** Ytterbium, thulium, erbium, terbium, and lutecium tracers were prepared by neutron irradiation of ca. 1 mg. of each of their oxides in the Argonne research reactor. In using the tracers, aliquots from stock solutions of the dissolved oxides were combined in a small beaker and the mixture was taken to dryness by heating under an infrared lamp. The residue was then taken up in 0.05M hydrochloric acid, and 30 to 40 µl. aliquots of this stock solution were used for each run for loading the column.
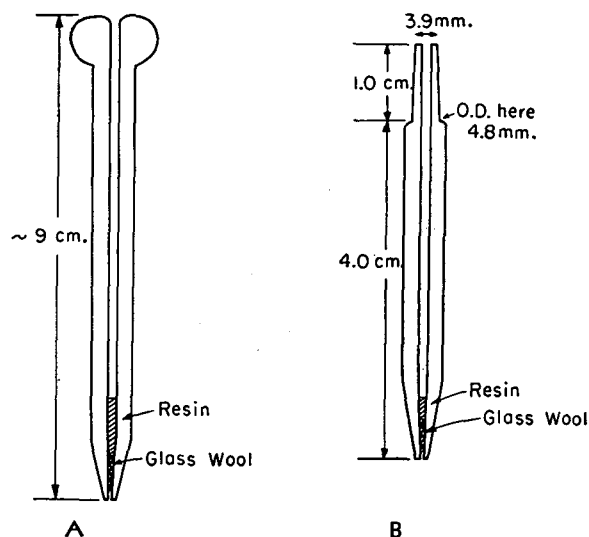


**Figure 1. Columns**

The experiments described in this paper were all performed days after the rare earth oxides were irradiated, so that the only radioactive nuclides present in the stock solutions of the tracers at the time were those having half lives of the order of days or longer. Decay curves taken on aliquots of the stock solutions during the interval of the experiments agreed with the assumption that the radioactive nuclides used were the 73-day terbium-160, the 9.4-day erbium-169 (with some 1.9-year thulium-171 present as a daughter of the 7.5-hour erbium-171), 129-day thulium-170, a mixture of 32-day ytterbium-169 and 4.2-day ytterbium-175, and the 6.8-day lutecium-177.

**Apparatus.** Columns of two different designs were used. The first was simply a 9-cm. length of capillary tubing 2 mm. in internal diameter having a male standard-taper 12/5 semiball joint at the top and a sharply drawn taper at the bottom (Figure 1, A). A glass wool plug in this tip held the resin in the column.

The experimental arrangement used with this column is diagrammed in Figure 2. A mercury-head pressure device, A, was connected by means of tubing to a rubber stopper in the top of a 50-ml. buret, B, which served as the eluting agent reservoir. The tip of the buret, in turn, was connected by means of Tygon tubing and a female 12/5 semiball joint to the column, C. Drops from the column tip fell through the light beam of a photoelectric drop counter, D, which activated the rotation of a 20-inch diameter turntable having 60 stainless steel counting disks 1 inch in diameter around its periphery. [This turntable (of $^1/_8$-inch Lucite sheet) was used with a chromatographic column fraction collector designed by Herman Robinson, Berkeley Radiation Laboratory. The drop counter modification was designed and built by M. T. Wiggins, Electronics Division, Argonne National Laboratory.] Usually in these runs the drop counter was set so as to collect one drop per disk.

The second column used in the present work (Figure 1, B) was designed to expedite its quick attachment to the rest of the system after loading with the material to be separated.

A 5-cm. length of precision-bore capillary tubing 2 mm. in internal diameter was drawn down at the tip as before, and the top was ground in a taper such that the column became interchangeable with the removable glass tip of an Ultramax buret (Emil Greiner Co., New York, N. Y.). In use, this buret was then set directly over the turntable of Figure 2. (A small amount of silicone stopcock grease on the upper tapered surface of the column aids in preventing leaks at the Teflon plug of the buret.)
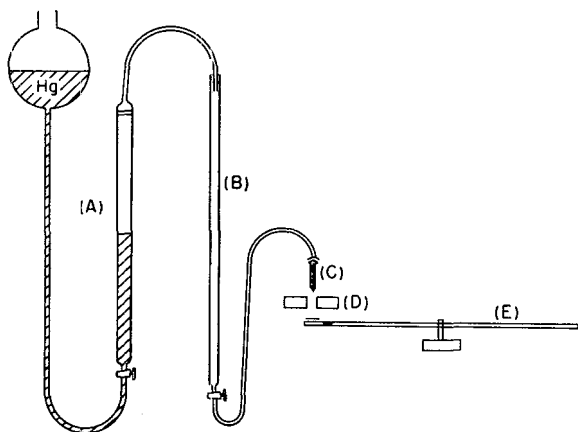


**Figure 2. Arrangement of columns**

**Run Procedure.** After a column had been loaded and attached to the rest of the assembly, a reading was taken of the eluting agent level in the buret. The desired pressure head was then applied, and the stopcock on the buret opened so that eluting agent passed through the column. For very rapid flow rates, the elapsed time during the run was measured with a stop watch; otherwise the times of opening and closing the buret were simply noted. At the end of the experiment, the pressure head was released and another buret reading taken. With these data, knowing the total number of drops passed, the mean drop size and mean drop time were readily calculated.

Drops on the disks were taken to dryness under an infrared lamp. Radioactivity measurements were ordinarily made without further treatment, as it was necessary only to outline the elution curve, and high counting precision was not required for the individual plates. Counting was done in a flow-type, end-window, proportional counter. Elution peak identifications were made by following the radioactive decay of plates taken at the peaks and by comparing these to similar plates made from the tracer stock

solutions. Absorption curves were taken if necessary for complete identification.

### RESULTS

**Minimum Column Size.** The 3 × 2 mm. column shown in Figure 1, B, was used and, because the resin of necessity was well down into the taper of the tip, the form of the column was actually that of a right conical section rather than a cylinder. The volume occupied by the resin was estimated as 5 ± 1 μl.

Figure 3 shows the separation obtained when thulium and terbium were the two ions present. The eluting agent for this run was 0.25M glycolate, pH 3.48, containing 0.05% Aerosol OT.

**Table I. Runs in 1-Cm. Column with pH 3.58 Glycolate**

| Run No. | Grade of Resin | Mean Drop Size, μl. | Mean Flow Rate, Ml./Sq.·Cm./Min. |
|---|---|---|---|
| 40 | 2–18-hour | 9.0 | 1.65 |
| 41 | 2–18-hour | 10.5 | 1.65 |
| 47 | 2–18-hour | 9.8 | 2.05 |
| 48 | 2–18-hour | 9.2 | 0.33 |
| 42 | 10-minute | 9.0 | 0.76 |
| 43 | 10–30-minute | 10.0 | 1.16 |
| 44 | 10–30-minute | 9.4 | 0.16 |
| 45 | 30-min.–2-hour | 9.5 | 1.10 |

**Runs at High Flow Rate.** Figure 4 shows the elution curves from two experiments where three out of four of the heaviest rare earths were largely separated from each other in less than 20 minutes. The column length was slightly less than 1 cm. for these experiments. Other operating data are given in Table I. (All of the Table I runs were made at room temperature with 0.25M glycolate, pH 3.58, containing 0.05% of Aerosol OT as the eluting agent.)
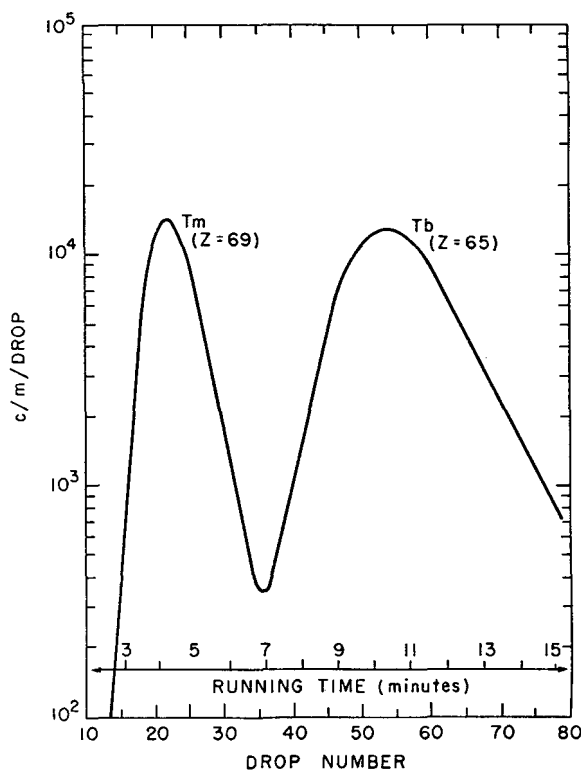


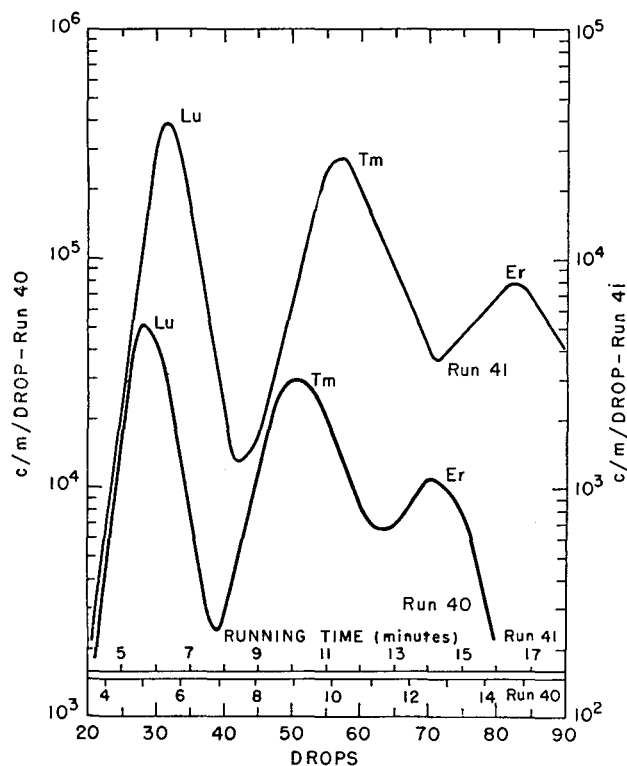**Figure 3. Separation of thulium and terbium by 3 × 2 mm. column**

Figure 4. Rapid separation runs

contain about 40 p.p.m. of calcium. It thus appeared that some of these very short resin columns had inadvertently been partially or wholly converted to the calcium form before use, thus explaining the erratic results obtained. In order to confirm this, a series of runs was made where the same 1-cm. length of 2- to 18-hour resin was deliberately converted to different chemical forms before an ytterbium-thulium separation was attempted, using pH 3.48, decationized 0.25$M$ glycolate without added wetting agent. Figure 7 gives typical elution curves for systems where the resin was initially in the ammonium, potassium, calcium, or aluminum form. Operating data are summarized in Table II.

### DISCUSSION

The curves of Figures 3 and 4 would indicate that tracer amounts of certain heavy rare earths can be very rapidly separated to a degree useful in radiochemical work by the use of very short resin columns with glycolic acid eluting solutions. Certain precautions must be observed: Preferably a very fine resin should be used, or, alternatively, a lower flow rate, with corresponding loss of speed in separation. The eluting solution should be treated to remove all cations save ammonium and hydrogen.

**Effect of Resin Size and Flow Rate.** For rare earth separations with cation resin columns it has been known for some time (6) that both slower flow rates and the use of finer resins will tend to give sharper peaks and better separations, as these factors are acting to allow column operation under more nearly equilibrium conditions. These points are illustrated for the glyolic acid–rare earth–Dowex 50 system in Figures 5 and 6. In the first of these, elution curves are given where the variable was the resin size (also compare to the lutecium thulium separation shown in Figure 4). In Figure 6, two pairs of elution curves are given where the effect of flow rate is shown. The operating data for all these runs are given in Table I. [As some of the curves of Figures 5 and 6 are offset for simplicity of presentation, no numerical values are given for the log scale of the ordinate. In Figure 6 the amount of eluating agent is expressed as the number of free column volumes passed as defined by Mayer and Tompkins (3).]



Figure 5. Effect of resin size

### Table II. Runs in 1-Cm. Column with pH 3.48 Glycolate

| Run | Initial Form of Resin | Mean Drop Size, $\mu$l. | Mean Flow Rate, Ml./Sq. Cm./Min. |
|---|---|---|---|
| 21 | $NH_4^+$ | 14.4 | 0.32 |
| 29 | $K^+$ | 15.7 | 0.41 |
| 23 | $Ca^{++}$ | 18.3 | 0.36 |
| 31 | $Al^{+++}$ | 15.9 | 0.59 |

**Effect of Resin Form.** At the time these very short column runs were started, considerable difficulty was met in obtaining reproducible elution curves. The eluting agent in use at that time had not been "decationized" by resin treatment, so when it was observed that the type of elution curve obtained depended on the length of time that the column was pretreated with the 0.25$M$ eluting solution, the latter was analyzed and shown to

The amount of acid in the solution used for adding the tracers must be kept as low as possible to avoid converting a substantial portion of the resin to the hydrogen form during the loading step. (This would have the effect of lowering the pH of the initial quantity of eluting agent as hydrogen ion is displaced by the ammonium present. This will cause a subsequent slowing down of the elution.)

On the basis of some other experiments (not presented here in detail) it seems inadvisable to use a 0.25$M$ eluting solution at pH levels much below 3.4, since the individual peaks are spread over a large number of drops and the length of the run is greatly increased.

The ultrashort columns were tested primarily with the nuclides enumerated above. In a few runs where americium and

Figure 6. Effect of rate of flow



Figure 7. Effect of initial form of resin in ytterbium-
thulium separation

curium were present, the separation of these two elements on the 1-cm. column was relatively poor, as was also the case with the terbium-yttrium pair. [Yttrium shows some interesting changes in position in various 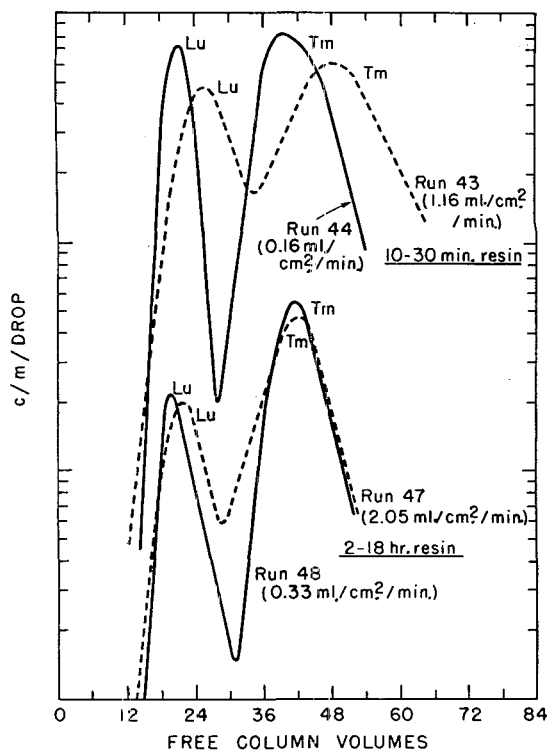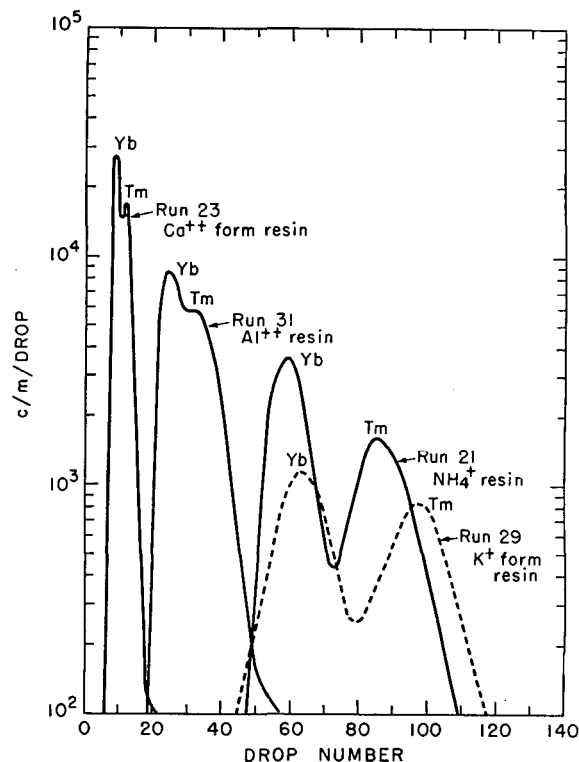elution systems. Ketelle and Boyd (1) found it between holmium and dysprosium when 5% citrate, pH 3.20, was used with ammonium form resin at 100° C. Spedding and Dye (4) found that with 5% citrate at all pH's at room temperature, the elution order was dysprosium-terbium-yttrium if hydrogen form resin was used; with the same form of resin, using 0.1% citrate at pH 5.5 to 6.0, the order became dysprosium-yttrium-terbium. In the present work, using columns of greater length than those reported here, the indications are that the order in glycolate systems with ammonium resin is dysprosium-terbium-yttrium.]

In some of the experiments reported here, surface active agents were used in the eluting solutions. This aids in obtaining a smaller drop size, as can be seen by contrasting the data of Tables I and II, and also makes it easier to obtain a fast flow rate when a very fine resin is being used. In some of the elution curves, an actual improvement in separation is apparently seen, but this is probably due only to the more precise delineation of the troughs between the peaks that results from the smaller drop size.

The curves of Figure 7 present a rather curious sequence. The marked similarity of the runs using ammonium and potassium form resins may be due simply to the similarity in the resin affinities of these two ions. Another possibility, in the case of the potassium column, is that the potassium ion is displaced from the resin by the mass action effect of the ammonium ion in the eluting solution, so that the rare earth bands "see" essentially nothing but an ammonium column. On the other hand, because the more highly charged calcium and aluminum ions have a greater affinity for the resin, the mass action effect of the ammonium ion in the eluting agent would not be so effective, so that the rare earth bands moving down the column are actually competing with calcium or aluminum ions for resin reactive positions. This would account for the fact that the rare earths

pass much more rapidly through the calcium and aluminum columns, although the order of that movement is the reverse of what might be expected—the doubly charged calcium apparently has more effect than the triply charged aluminum. This may be due to the fact that aluminum ion itself is probably complexed to a certain extent by glycolate and is thus more readily removed from the resin.

The separations reported by Mayer and Freiling would indicate that at 87° C., glycolic acid as an eluting agent for the rare earths offers about the same degree of improvement over citric acid as does lactic acid. Like lactic acid, and in contrast to citrate systems, the glycolic acid eluting agents are much less pH-sensitive, thus permitting a wider range of simply attained conditions for meeting specific separations problems. One definite advantage of glycolic acid over either of the others is its higher stability in solution. Stocks of the acid have been left at room temperatures for weeks with no change in pH or in apparent effectiveness as an eluting agent.

### ACKNOWLEDGMENT

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# Determining Trace Metals in Petroleum Distillates

## An Acid-Extraction Technique

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Catalytic refining of petroleum demands a knowledge of minute traces of metal compounds that occur in petroleum fractions. Metals analyses that involve combustion of the oil may yield low results because of volatilization and entrainment. An acid-extraction technique has been developed for separating these metals from the oil, after which they are determined by spectrographic analysis. The oil is extracted first with sulfuric acid and then with a mixture of hydrochloric acid, acetone, and water. After concentration, the extract is transferred to graphite electrodes for spectrographic analysis in a direct-current arc. If spectrographic equipment is not available, sensitive chemical methods can be used. The coefficient of variation of the method is 11%. About 6 man-hours and 72 hours of elapsed time are required per analysis. Six methods for recovering copper, iron, lead, nickel, and vanadium from a gas oil have been compared with the acid-extraction technique: simple ashing, partial sulfated ashing, total sulfated ashing, extraction with iodine, extraction with a mixture of hydriodic and acetic acids, and extraction with a mixture of hydrobromic and acetic acids. Total sulfated ashing, extraction with hydrobromic and acetic acids, and acid extraction recovered the five metals quantitatively. The other four methods were unsatisfactory. Partial sulfated ashing gave no better results than simple ashing.

THE increased use of catalytic processes in the refining of petroleum has intensified the study of trace metals occurring in petroleum fractions. These metals may have been present in the original petroleum or introduced during prior processing. Only a few tenths of a part per million of such metals as copper, iron, lead, nickel, and vanadium in the feed may decrease catalyst activity. Knowledge of the concentrations of these metals is thus important to the refiner.

Many schemes have been devised for the determination of metals in petroleum fractions. Some cover only concentrations above 1 p.p.m.; others extend the detectable limit to 0.1 p.p.m. Copper, iron, nickel, and vanadium have been determined in crude oils by micropolarographic and colorimetric techniques in concentrations as low as 0.1 p.p.m. (19). Colorimetry has also been applied to iron, nickel, and vanadium in petroleum fractions (29) and vanadium in fuel-oil ash (13, 14).

The emission spectrograph offers several advantages over these techniques. If the metals content is high enough, the oil can be analyzed directly without prior ashing. Several spectrographers have directly determined metals in lubricating oils (3, 4, 7, 10, 22). A cathode-layer technique (15) has been used in the direct analysis of heavy petroleum stocks. Key and Hoggan (16) used a nitrogen atmosphere to spark residual fuels. These procedures are satisfactory for crude oils, residual fuels, and other fractions containing many parts per million of metals, but they are not sensitive enough for gas oils and naphthas. Concentration of the metals before measuring the amounts present is therefore necessary.

Even when direct analysis of the oil is not feasible, spectrographic methods can be carried out upon ashed residues in less time than chemical methods. No separations are required, and the metals are determined simultaneously. A semiquantitative

method for determining metals in ashed residues has been devised by Murray and Plagge (20). Three quantitative methods have recently been proposed (1, 5, 14). In all four methods, ashing the oil may lead to loss of metals by volatilization and entrainment (15, 19). Chemical oxidation of the oil would eliminate these difficulties, but it requires large amounts of mineral acids and is slow.



**Figure 1. Extraction apparatus**

A new extraction technique has been developed that eliminates ashing of the oil and does not require large amounts of mineral acids. As little as 0.01 p.p.m. of some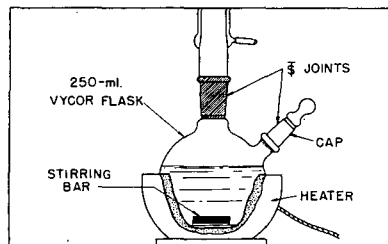 metals can be determined in only 50 grams of oil. The oil is extracted first with concentrated sulfuric acid and then with a mixture of concentrated hydrochloric acid, acetone, and water. The extract is evaporated to dryness, the carbonaceous material is removed by ignition, and the inorganic residue is dissolved in hydrochloric acid. The acid solution is evaporated to about 0.2 ml. and transferred to graphite electrodes for spectrographic analysis in a direct-current arc. Metals in the oil are determined from working curves based on standardized aqueous solutions. If spectrographic equipment is not available, the acid solutions may be analyzed by any sensitive chemical procedure.

## EQUIPMENT

For extraction of the oil, a 300-ml. two-necked flat-bottomed flask, especially constructed of Vycor brand glass (96% silica), and a 30-cm. reflux condenser are used. The extraction flask is shown in Figure 1. The stirring bar is covered with Vycor and inserted in a Teflon sleeve.

Spectrographic equipment consists of a Bausch and Lomb large Littrow spectrograph, a Multisource power supply, an A.R.L.-Dietert comparator-densitometer, and other conventional equipment. Table I summarizes the excitation conditions and other pertinent spectrographic information. A two-step adjustable sector is inserted in the optical path to provide copper lines of

**Table I. Summary of Spectrographic Variables**

| | |
|---|---|
| A.c. input, volts | 230 |
| D.c. output, volts | 300 |
| Capacitance, μfd. | 60 |
| Inductance, μh. | 480 |
| Resistance, ohms | 18 |
| Exposure, seconds | 60 |
| Slit width, microns | 20 |
| Wedge, mm. | 5 |
| Analytical gap, mm. | 9 |
| | |
| Upper electrodes | Undercut center-post |
| Lower electrodes | Flat chamfered |
| Internal standard | Cobalt |
| Emulsion | S.A.-2 |
| Developer | D-19 |
| | |
| Development, min. | 3 |
| Acid stop, sec. | 20 |
| Fix, min. | 5 |
| Wash, min. | 10 |
| Drying, min. | 5 |

suitable intensity. The photographic emulsion is calibrated by standard methods (12).

## REAGENTS

All mineral acids used in the extraction are of microanalytical grade. Other chemicals are reagent grade. All reagents and distilled water were checked for impurities by spectrographic analysis, and those showing the least impurities were selected.

The reference solution contains copper, iron, lead, manganese, nickel, sodium, vanadium, and zinc in concentrations of 0.100 mg. per ml. It is prepared from stock solutions of the metal chlorides or nitrates, standardized by any convenient procedure.

A solution containing 0.02 mg. of cobalt per ml. supplies the internal-standard element. It is prepared by diluting the correct amount of 6% Hexogen cobalt with a highly refined mineral oil.

## PROCEDURE

A representative 50-gram portion of the oil, warmed if necessary, is weighed into the extraction flask. Fifty milliliters of n-heptane and 1.00 ml. of the internal-standard solution are added, and stirring is begun. Five milliliters of concentrated sulfuric acid is then added, and the mixture is refluxed 1 hour at about 100° C., as governed by n-heptane reflux. The oil layer is decanted, and the acid layer is transferred to a 250-ml. Vycor Erlenmeyer flask.

The oil layer is returned to the extraction flask and refluxed 1 hour at 100° C. with 50 ml. of a mixture containing 2 parts of concentrated hydrochloric acid, 1 part of acetone, and 1 part of distilled water. The oil-acid mixture is transferred to a separatory funnel and shaken thoroughly. After the aqueous layer settles, it is removed and added to the Erlenmeyer flask. The remaining oil is washed with 20 ml. of a 50 to 50 mixture of acetone and distilled water, and the washings are added to the Erlenmeyer flask. Any asphaltic residues remaining in the extraction flask are removed by several rinses with 10-ml. portions of acetone and added to the acid extracts.

To the combined extracts is added 2 ml. of concentrated sulfuric acid. The mixture is heated progressively until all the acetone, water, hydrochloric acid, and sulfuric acid have been removed. Care should be exercised to avoid superheating. The residue is ignited at 550° C. until all carbonaceous material has been removed. The remaining inorganic material is taken up in a minimum amount of hydrochloric acid and concentrated to about 0.2 ml. A flat chamfered 1/4-inch graphite electrode is sealed by allowing it to soak up one drop of mineral oil under an infrared lamp. The hydrochloric acid concentrate is evaporated in increments on the electrode under the infrared lamp. The mineral oil is removed by heating the electrodes in ashing coils (2). The deposits are subjected to spectrographic excitation using a direct-current arc.

To allow correction for metal impurities in the reagents and distilled water, a blank is prepared by carrying out the extraction procedure without oil.

To prepare calibration standards, 1 ml. of the cobalt solution is oxidized with 1 ml. of concentrated sulfuric acid, ignited to remove the organic residue, and taken up in 1 ml. of concentrated hydrochloric acid. The proper amount of the reference solution is added to give a series of standards representing 0.02 to 5.0 p.p.m. of the various elements in terms of a 50-gram sample of oil. These solutions are treated in the same manner as the hydrochloric acid solutions of the ashed residues.

Analytical working curves are prepared by plotting the parts per million of the elements represented by each standard against the corresponding ratio of the intensity of the spectral lines listed in Table II to the intensity of the cobalt internal-standard line. If a 50-gram sample of oil is being analyzed, concentration in parts per million can be read directly from these curves.

## DISCUSSION

Completeness of extraction was demonstrated by carrying out semiquantitative analyses (11) of both the extract and the extracted oil for several different oils. Photometering of suitable spectral lines for the metals present showed that more than 95% of the copper, iron, lead, manganese, nickel, and vanadium were extracted from the oil. Sodium and zinc were not present in the oils in significant amounts; however, these two elements are easily removed from petroleum fractions.

The precision of the procedure was established by triplicate analyses of two different oils. The results are shown in Table III. When applied to the determination of copper, iron, lead, nickel, and vanadium, the procedure shows a coefficient of variation of 11%.

### Table II. Spectral Lines Used for Analysis

| Element | Concn. Range, P.P.M. | Line, A. |
|---|---|---|
| Copper | 0.02–0.5 | 3274.0 [a] |
| | 0.5 –5.0 | 2618.4 |
| Iron | 0.05–1.0 | 2973.2 |
| | 0.5 –5.0 | 2912.2 |
| Lead | 0.02–0.2 | 2833.1 |
| | 0.2 –5.0 | 2663.4 |
| Manganese | 0.02–0.5 | 2801.1 |
| | 0.2 –5.0 | 2925.6 |
| Nickel | 0.02–0.5 | 3003.6 |
| | 0.05–2.0 | 2992.5 |
| | 1.0 –5.0 | 2821.3 |
| Sodium | 1.0 –5.0 | 5895.9 |
| Vanadium | 0.02–0.5 | 3056.3 |
| | 0.2 –5.0 | 3198.0 |
| | 0.2 –5.0 | 2914.9 |
| Zinc | 0.1 –5.0 | 3345.0 |
| Cobalt [b] | .... | 3044.0 |

[a] Sectored line.   [b] Internal-standard line.

### Table III. Triplicate Analyses of Two Petroleum Fractions

(Parts per million)

| | | Cu | Fe | Pb | Ni | V |
|---|---|---|---|---|---|---|
| Mid-continent gas oil | | 0.22 | 0.51 | 0.73 | 0.61 | 1.75 |
| | | 0.18 | 0.35 | 0.68 | 0.72 | 1.23 |
| | | 0.15 | 0.44 | 0.66 | 0.64 | 1.65 |
| | Av. | 0.18 | 0.43 | 0.69 | 0.66 | 1.54 |
| Boscan gas oil | | 0.68 | 1.03 | 0.48 | [a] | 0.21 |
| | | 0.78 | 0.92 | 0.44 | [a] | 0.18 |
| | | 0.85 | 0.95 | 0.52 | [a] | 0.18 |
| | Av. | 0.77 | 0.97 | 0.48 | | 0.19 |

[a] Too low for accurate analysis.

### Table IV. Analysis of an Enriched Gas Oil

| Element | Parts per Million | |
|---|---|---|
| | Added | Found |
| Copper | 2.45 | 2.20, 2.05 |
| | | 2.25, 1.85 |
| Iron | 2.50 | 2.34, 2.74 |
| | | 2.19, 2.74 |
| Lead | 2.50 | 2.91, 2.64 |
| | | 2.66, 2.01 |
| Vanadium | 2.50 | 2.43, 2.63 |
| | | 2.68, 2.58 |

Accuracy of the procedure was estimated by analyzing an oil containing known added amounts of four elements. A mid-continent gas oil was enriched with mineral-oil solutions of copper, iron, lead, and vanadium naphthenates that added 2.5 p.p.m. of each element. Both the original and the enriched gas oil were analyzed by the extraction procedure, and the difference between the two values was calculated. Results are shown in Table IV. On the average, a single analysis is within 12% of the true value.

The sensitivity of the procedure is governed by the ratio of the concentration of a given element in the oil to that in the blank. Hence, the sensitivity for any element may be increased by analyzing a larger sample. Fifty grams was chosen as a compromise between ease of handling and sensitivity. With this sample size, the sensitivity for eight metals that often must be determined in gas oils is:

| Element | Cu | Fe | Pb | Mn | Ni | Na | V | Zn |
|---|---|---|---|---|---|---|---|---|
| Limit, p.p.m. | 0.02 | 0.05 | 0.02 | 0.01 | 0.01 | 1.0 | 0.01 | 0.1 |

Other metals may be determined if they are included in the standards. Silicon and magnesium cannot be determined because they always are found in large amounts in the blank.

## CONCLUSION

The extraction procedure is particularly well suited to the analysis of petroleum fractions containing less than 0.5 p.p.m. of metals. About 6 man-hours and 72 hours of elapsed time are required per analysis. Although the procedure is long, it is shorter than other procedures giving comparable results. Sensitive methods other than spectrographic may also be used for the analysis of the acid extracts.

# (Determining Trace Metals in Petroleum Distillates)

## Efficiency of Recovery Methods

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METALS that may be present in petroleum distillates as metallo-organic compounds, especially porphyrins, are recovered from the oil only with great difficulty (*18, 25, 27, 28*). These compounds usually present the greatest problem in recovering the metals from oil.

Ashing and extraction are the usual schemes for concentrating the metals, although other techniques have been proposed (*6*). The simplest and most direct ashing technique is burning the oil and igniting the residue until only an inorganic ash remains (*20*). To prevent losses of metals by volatilization or entrainment, investigators have suggested sulfation of the ash at some step during combustion (*15*). Sulfation of the oil without combustion has also been proposed (*19*). Extraction media suggested, other than hydrochloric and sulfuric acids, are pentane (*24*), acetic acid (*21*), hydrochloric acid (*8, 17, 23*), and hydrogen halides and acetic acid (*9, 26, 27*).

Six methods showing promise for the quantitative recovery of metals from a gas oil were compared with total sulfated ashing, a method which should recover all the metals present. They included simple ashing, partial sulfated ashing, extraction with iodine, extraction with a mixture of hydriodic and acetic acids, extraction with a mixture of hydrobromic and acetic acids, and acid extraction.

### REAGENTS AND EQUIPMENT

The petroleum fraction taken for analysis should contain a high concentration of contaminating metals to test the separations methods as rigorously as possible, and it should also contain porphyrins to provide the most severe test. A gas oil cut from a Boscan (Venezuela) crude oil was considered the best choice. Semiquantitative spectrographic analysis (*11*) showed that it contained between 0.1 and 1.0 p.p.m. of vanadium. The oil was examined for porphyrins by diluting with benzene and extracting with a hot, saturated solution of hydrobromic acid in acetic acid. The acid layer was removed, the oil layer was extracted with 20% hydrochloric acid, and the two acid layers were combined. Any porphyrins present in the acid phase were extracted (*9*). An absorption spectrum of a chloroform solution of the final extract is shown in Figure 2. The two peaks at 497 and 565 m$\mu$ coincide with those reported for vanadium porphyrins (*9*).

Microanalytical grade mineral acids were used for extraction and sulfation procedures; reagent grade chemicals were used for all standard solutions and extractions not requiring mineral acids.

Two special solutions and six reference solutions were required. Acetic acid saturated with hydrobromic acid was prepared by bubbling anhydrous hydrogen bromide through glacial acetic acid in an ice bath; this solution is highly corrosive. Acetic acid saturated with hydriodic acid was prepared by passing anhydrous sulfur dioxide through a mixture of 100 ml. of glacial acetic acid, 10 grams of iodine, and just enough water for the stoichiometric reduction of iodine to hydriodic acid. Solutions containing 10.0 mg. of the metal per ml. were prepared from cobalt, copper, ferric, lead, nickel, and vanadyl nitrates. A solution containing 0.100 mg. of cobalt per ml. as cobalt nitrate supplied the internal-standard element.

Spectrographic equipment and electrodes were as described above.

### SEPARATION OF METALS

Three ashing and four extraction methods were carried out. One hundred grams of oil were treated. Each method produced a carbonaceous residue that was ignited at 550° C. to an inorganic residue.

**Simple Ashing.** For simple ashing, the gas oil was weighed into a platinum evaporating dish, placed on a hot plate, and heated until it was readily ignited by a Bunsen flame. The oil was permitted to burn until only a carbonaceous residue remained.

**Partial Sulfated Ashing.** Partial ashing resembled simple ashing in that the oil was first burned without pretreatment. The carbonaceous residue was then treated with 5 ml. of concentrated sulfuric acid and heated to drive off the excess sulfur trioxide.

**Total Sulfated Ashing.** For total sulfated ashing, the oil and 50 ml. of concentrated sulfuric acid were mixed thoroughly in a Vycor beaker. The water and excess acid were removed by heating cautiously on a hot plate with intermittent stirring.

**Extraction with Iodine.** The oil and 10 grams of iodine were heated in a Vycor beaker on a hot plate at 120° C. for 24 hours with occasional stirring. After cooling, the bulk of the oil was decanted, and 2 ml. of concentrated sulfuric acid was added to the plastic semisolid residue. The mixture was heated until the excess sulfur trioxide was driven off.

**Extraction with Hydriodic Acid and Acetic Acid.** The oil and 25 ml. of the saturated solution of hydriodic acid in glacial acetic acid were refluxed at 40° to 50° C. for 72 hours with continuous stirring. The supernatant liquid was decanted, and to it was added 25 ml. of equal parts 20% hydrochloric acid and acetone.
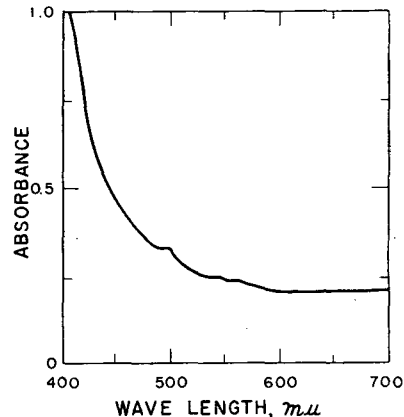


**Figure 2. Absorption curve of porphyrin extract from Boscan gas oil**

This solution was shaken thoroughly and the acid layer was removed. The oil layer was washed with 25 ml. of the mixture of hydrochloric acid and acetone, and the acid layer was removed. The material remaining in the extraction flask was combined with the other acid layers, 2 ml. of concentrated sulfuric acid was added, and the solution was heated on a hot plate to drive off the excess sulfur trioxide.

**Extraction with Hydrobromic Acid and Acetic Acid.** The oil, 50 ml. of benzene, and 50 ml. of the saturated solution of hydrobromic acid in glacial acetic acid were refluxed at 40° to 50° C. for 24 hours with continuous stirring. To the hot liquid was added 25 ml. of equal parts of 20% hydrochloric acid and acetone, and the acid layer was removed. The remaining solution was washed with 25 ml. of the hydrochloric acid–acetone mixture, and the two layers were combined. After addition of 2 ml. of concentrated sulfuric acid, the acid extracts were heated on a hot plate to drive off the excess sulfur trioxide.

**Extraction with Sulfuric Acid and Hydrochloric Acid.** The procedure described above was used, except that the internal-standard solution was not added to the oil.

## SPECTROGRAPHIC ANALYSIS OF RESIDUES

All inorganic residues were dissolved in hot hydrochloric acid, treated with 0.200 ml. of the cobalt nitrate solution, and concentrated to about 0.2 ml. These concentrates were evaporated on flat chamfered electrodes that had previously been sealed with white oil. The inorganic deposits were then subjected to spectrographic excitation under the previously described conditions.

To prepare calibration standards, the proper amounts of the reference solutions were aliquoted to give a series of standards representing 0.02 to 5.0 p.p.m. of copper, iron, lead, nickel, and vanadium, in terms of a 100-gram sample of oil. To each standard were added 0.500 ml. of the cobalt nitrate internal-standard solution and the same amount of concentrated hydrochloric acid that was used to dissolve the inorganic residues. The solutions were then treated in the same manner as the hydrochloric acid solutions of the ashed residues.

After the photographic plates were processed, the spectral lines for cobalt, copper, iron, lead, nickel, and vanadium included in Table II were photometered. Working curves were prepared as described in the procedure.

## RESULTS AND DISCUSSION

Spectrographic results are presented in Table V. Each value is the average of two or three individual analyses.

**Table V. Spectrographic Analysis of Gas-Oil Residues**

| Method of Separation | (Parts per million) | | | | |
|---|---|---|---|---|---|
| | Cu | Fe | Pb | Ni | V |
| Ashing | | | | | |
| Simple | 0.030 | 0.93 | 0.20 | 0.023 | 0.18 |
| Partial sulfated | 0.048 | 0.55 | 0.18 | 0.022 | 0.17 |
| Total sulfated | 0.051 | 0.92 | 0.38 | 0.023 | 0.18 |
| Extraction | | | | | |
| Iodine | a | a | 0.33 | a | 0.16 |
| HBr-CH₃COOH | 0.055 | 0.93 | 0.38 | 0.021 | 0.17 |
| HI-CH₃COOH | 0.01 b | 0.48 | 0.24 | 0.015 b | 0.16 |
| H₂SO₄-HCl | 0.050 | 0.92 | 0.38 | 0.022 | 0.19 |

a Blank too high to permit accurate measurement.
b Maximum values.

If total sulfated ashing is selected as the basis of comparison, four observations apply to the other procedures. Only acid extraction or extractions with hydrobromic and acetic acids recover the five metals quantitatively. Extraction with hydriodic and acetic acids recovers most of the vanadium and lesser amounts of the other metals. Extraction with iodine is completely unsatisfactory. Because neither simple ashing nor partial sulfated ashing recovers lead quantitatively, the loss seems to occur during combustion rather than ignition.

These observations are valid only for the gas oil studied. Some nickel, vanadium, and perhaps iron may be lost during combustion of other petroleum fractions. Loss of metals by entrainment or by incomplete separation in acid extraction may depend on the state of combination of the metals in the oil, the boiling range of the oil, and the concentration of the metals themselves.

Simple ashing should not be indiscriminately used in determining trace metals in petroleum fractions. It should only be used in comparing similar samples where speed is of primary importance. If a series of like samples is to be analyzed, the amounts of the various metals lost by simple ashing may be determined once by comparison with a more accurate procedure and corrected for.

Of the two methods giving quantitative results, extraction with sulfuric acid and hydrochloric acid is better than extraction with hydrobromic acid and acetic acid. As a routine method it is less hazardous and faster, and physical separation of the oil

and acid layers is easier. It is somewhat faster than total sulfated ashing, and is more reliable because of smaller corrections for blanks.

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# Determination of Low Alkalinity or Acidity in Water—Correction

In the article, "Determination of Low Alkalinity or Acidity in Water" [Larsen, T. E., and Henley, Laurel, ANAL. CHEM., 27, 851 (1955)], the acknowledgment in the first sentence of the second paragraph is incorrect. The data reported in Figure 2 were obtained on surplus portions of samples collected in connection with a subcontract with the Cloud Physics Project of the University of Chicago, a research project sponsored by the Geophysics Research Directorate of the Air Force Cambridge Research Center, Air Research and Development Command, under Contract AF19(604)-618. The authors wish to express their thanks for the privilege of using these samples.

T. E. LARSEN
LAUREL HENLEY

# Rotational Viscometer for Rapidly Settling Suspensions

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A simple, sensitive, and sturdy rotational viscometer of the concentric cylindrical type has been developed to determine the apparent viscosities of some quickly settling suspensions of finely powdered solids in liquids. The essential features of this design include continuous operation at different speeds, provision for draining out of contents, and a simple mechanical arrangement to measure the torque developed on the stationary cylinder. The viscometer was calibrated against standard glycerol-water solutions, the determinations being carried out in the turbulent range, and the data correlated as a plot of specific friction factor vs. specific Reynolds number representing the calibration curve for this instrument. The design of the instrument, calibration with some liquids of known viscosity, and its application for the measurement of apparent viscosity of some rapidly settling suspensions are presented.

IN THE course of studies on the flow of solid-in-liquid suspensions in vertical columns (2), great difficulty was experienced in determining the apparent viscosities of some slurries in which there was rapid settling out of the finely divided solids from the liquid medium. Trials in rotational viscometers of the Couette type (3) with the cup as the rotating member proved unsuccessful, owing to quick phase separation under the influence of centrifugal force. Attempts were made to determine viscosity in capillaries by allowing a constantly stirred slurry to flow through a known vertical length by siphoning technique. The time required for the flow of a definite volume of slurry was determined and the value of viscosity was calculated in the usual way. The results did not show any marked deviation from that obtained in the rotational viscometer; however, this method was regarded theoretically unsound and involved considerable experimental difficulties, particularly with slurries of high solid content.

It was, therefore, found necessary to design and construct a continuously operating concentric cylindrical viscometer in which the inner cylinder, driven by a motor, acted as a stirrer. The value of viscosity was determined from the measurement of torque developed on the outer cylinder, by a simple mechanical arrangement.

## THEORY

Rotational viscometers are fundamental in nature and permit relative as well as absolute measurement of viscosity of fluids. Such instruments essentially consist of two concentric cylinders—the outer cup or container, and the inner spindle or bob. Theoretically it is immaterial which of the cylinders is rotated, but in practice the choice depends on the magnitude of the couple generated—that is, the value of viscosity.

The determination of apparent viscosity of quickly settling suspensions is a difficult problem, as none of the conventional rotational viscometers are suitable for this purpose. Wilhelm and others (6), however, designed an instrument in which it was possible to prevent the settling of the solids in suspension by providing paddles at the bottom of the rotating inner cylinder and baffles on the wall of the stationary container. They determined the flow properties of concentrated suspensions of cement rock and Filter-cel in water in the viscous as well as turbulent range.

In viscometers with stationary cup and rotating spindle, the inner cylinder is driven by a predetermined torque delivered by a system of weights and pulleys, and the number of revolutions is noted when a steady state is attained. The value of viscosity of fluid is obtained from the relation (1)

$$\eta = \frac{(W - w) R}{4\pi h\Omega} \frac{(r_2^2 - r_1^2)}{r_1^2 \, r_2^2} \quad (1)$$

where $W$ is the applied load, $w$ is the equivalent of the friction in the rotating system, $R$ is the radius of the pulley, and $h$ is the length of the inner cylinder of radius $r_1$ inside a container of radius $r_2$ rotating at uniform angular velocity $\Omega$.

Owing to the presence of paddles and baffles which give rise to end effects, viscosities cannot be calculated from the speed, torque, and dimension data using Equation 1, which in the simplified form is

$$T = k\mu N \quad (2)$$

This difficulty can be overcome by using the method developed by Squires and Dockendorff (5) for the evaluation of viscosity in rotational viscometers when the motion of the fluid is turbulent. In this procedure, a calibration curve is made for a specific instrument in terms of friction factor vs. Reynolds number where the friction factor is defined as

$$f = \frac{T}{16 \, r_1^2 r_2^2 h\rho N^2} \quad (3)$$

By analogy with flow of fluids in pipes, $f$ is a function of Reynolds number, Re, which can be defined as

$$Re = \frac{4N\rho \, (r_2^2 - r_1^2)}{\mu} \quad (4)$$

This means $f = \varphi \, (Re)$ $\quad (5)$

where $\varphi$ represents the functional relationship.

In the viscous region the slope of the curve, obtained by logarithmic plot of torque against speed, is unity and therefore the relation becomes simple and reduces to

$$f = \frac{\pi}{Re} \quad (6)$$

In the turbulent range, for a given fluid, however, there will be only one value of viscosity corresponding to any given value of $f$ and Re—that is, to any given value of $N$.

In instruments where there are baffles, paddles, etc., a new friction factor term

$$f' = \frac{T}{\rho N^2} \quad (7)$$

and a new Reynolds criterion

$$Re' = \frac{N\rho}{\mu} \quad (8)$$

have been defined. The terms $f'$ and $Re'$ are proportional to friction factor and Reynolds number, respectively, and the constants of proportionality are dependent on the design of the instrument.

The quantities $f'$ and $Re'$ are determined for fluids of known viscosity in the turbulent region and a plot correlating these is used as the calibration curve for the particular instrument. Now, from the measurement of torque at a particular speed, $f'$ for a given fluid can easily be calculated. The turbulent viscosity is then evaluated from $Re'$, obtained from the calibration curve, corresponding to this value of $f'$.

In the present paper are described the design and calibration of a rotational viscometer of the rotating spindle type in which the outer cylinder is also free to move. The torque exerted on the outer cylinder sets it in motion, which, however, is simultaneously counteracted by a calibrated watch spring. This brings the cylinder to rest. From the degree of twist of the spring the viscosity values are calculated. A few typical data on the apparent viscosities of some quickly settling suspensions are also presented.

## DESCRIPTION OF VISCOMETER

The viscometer is shown in detail in Figure 1.

The instrument essentially consists of two brass cylinders, A and B. The outer cylinder, B, of internal diameter of 2 inches and internal height of 4$^9$/$_{16}$ inches serves as the container. It has a working capacity of about 100 cc. and is provided with a hollow stem, C, to drain out the contents. The opening is closed by a small plug, D. This cylinder is supported by two extra light ball bearings (E.L. 9 A.), E, F, lodged in two steel housings, G, H. These housings are fitted in circular spigots and held together by three screws. The supporting casing, I, is fixed to the bottom plate, J, along with G and H by three screws. The drive shaft of the inner cylinder is fitted in the ball bearing, K, cased in the steel housing, L, which rests on I. The purpose of the supporting case, I, is to hold in position the bearing housings, fitted to the inner cylinder shaft on the top and the outer cylinder at the bottom, in specially made circular spigots to secure the concentricity of the cylinders. Six symmetrical holes are provided in L, which permit introduction of the test fluid and allow inspection during operation. The inner bob, A, of diameter of 1.5 inches and height of 3 inches is hollow but closed at both ends; two small paddles, M$_1$, M$_2$, are fitted at the bottom to keep the solids in suspension by stirring action.

The inner cylinder is directly coupled to a $^1$/$_{30}$-hp. Kestner alternating-direct current motor, which is fixed on the top
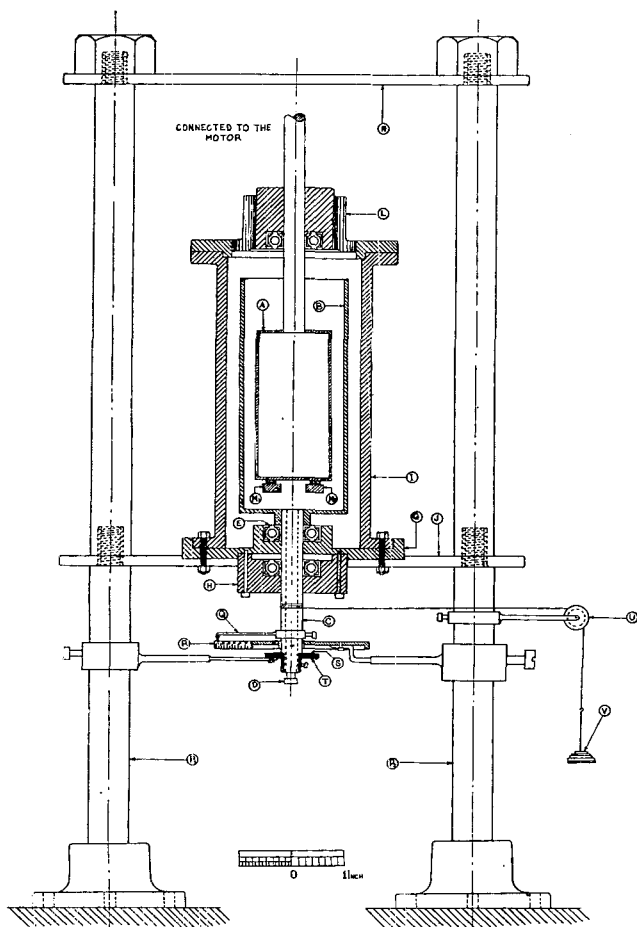
triangular plate, N. The instrument is provided with three supporting legs, P$_1$, P$_2$, P$_3$, which are extended by mild steel rods to hold the triangular plates, J and N, in position. The legs are rigidly fixed to the working table to prevent any vibration during operation.

The motor is connected to a constant 110-volt alternating current supply through a 230-volt stepdown transformer with a voltage regulator from the mains. The speed of the motor, controlled by an external Variac, is measured by a tachometer having a range of 0 to 1000 r.p.m. The tachometer is carefully mounted on the top of the motor with the help of a specially made fixture provided with springs to absorb any sudden shock during the speed measurement. The revolutions per minute of the motor can be noted whenever required by pressing the release key of the tachometer.

The motor with the top plate and the inner cylinder is removable. This permits ready access to the outer cylinder for cleaning and refilling.
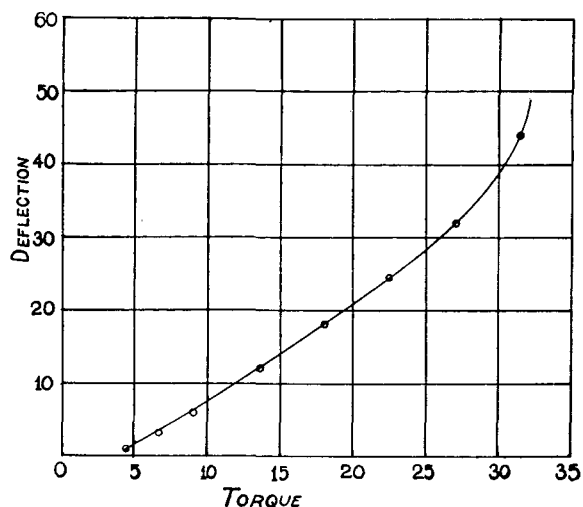


**Figure 2. Spring calibration diagram deflection**

Deflection. 1 unit = 4°

The light pointer, Q, attached to the stem, C, can move over a circular graduated disk, R. The scale is divided into 180 equal divisions, each representing 2°. A spiral spring, S, Roscoe watch spring No. 13, is housed in a suitable casing, T. The outer end of the spring is fixed to the inner wall of T while the inner end is attached to a hook on a small collar fitted to the stem, C, by a small screw. The spring housing is held in position by means of a brass rod fastened to one of the legs.

## CALIBRATION OF SPRING

The method of calibration is simple and direct. The torque required to turn the spring through a number of degrees is measured as on an analytical balance. This is done by attaching a string to the stem of the outer cylinder and running it over a small pulley fixed to a stand, U. The string carries a small pan, V, similar to a balance pan, at its lower end. Known weights, placed on the pan, cause the spring to twist through a certain number of degrees. The torque produced is the product of the radius of the stem, C, and the weight. A plot of torque against spring deflection is approximately linear up to a certain range and beyond that there is sharp deviation (Figure 2). The working range for a particular spring should be limited to the linear portion of the plot. To extend the working range of the instrument watch springs of different stiffness may be used.

## PROCEDURE AND RESULTS

The instrument was initially calibrated with liquids of known viscosity. A definite volume of liquid was introduced in the outer cylinder after plugging the drain hole. The motor was brought to several speeds by means of the external Variac and the revolutions per minute were noted by means of the tachometer. The degree of rotation of the outer cylinder corresponding to a particular speed of the inner spindle was recorded from



**Figure 1. Sectional view of viscometer**

the deflection of the pointer on the graduated scale. Experiments were performed in the speed range of 400 to 1000 r.p.m. The scale readings were reproducible within ±1° C. Most of the measurements were carried out at a temperature of 30° ± 0.2° C. The liquids employed for calibration were water and three water-glycerol solutions which covered the required range of 1 to 5 centipoises.

In order to determine the viscosity of the glycerol water solutions, Ostwald-Fenske viscometers Nos. 50 and 100 were used. The Ostwald readings were checked with water at 30° ± 0.1° C.



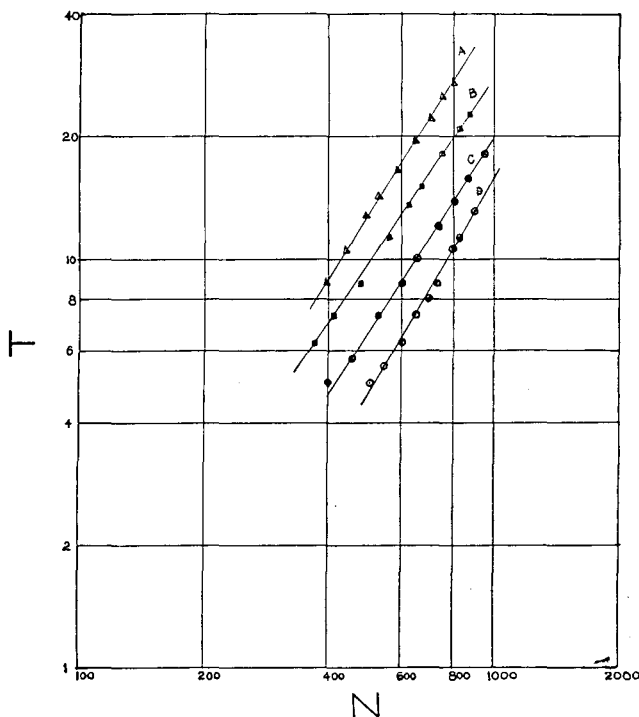Figure 3. Torque–r.p.m. relations for glycerol-water solutions at 30° C.

| | Density, Grams/Cc. | Viscosity, Centipoises |
|---|---|---|
| A | 1.125 | 4.315 |
| B | 1.090 | 2.650 |
| C | 1.046 | 1.360 |
| D | 0.996 | 0.800 (water) |

A logarithmic plot of torque readings against revolutions per minute was made as shown in Figure 3. It will be seen that these curves have slopes greater than unity. It was, therefore, evident that the determinations were carried out in the turbulent range. Hence, the values of $f'$ and Re' were calculated for several values of $N$ from the knowledge of density and viscosity data using Equations 7 and 8. On plotting $f'$ against Re' a straight line was obtained correlating viscometer data for glycerol-water solutions and water. This represented the calibration curve for the instrument (Figure 4). The above linear relation was possible to obtain because of the narrow experimental range with specific Reynolds number varying from 100 to 1000.

To determine the viscosity of unknown liquids and suspensions, a measured volume was introduced and the inner cylinder was rotated at several speeds and the pointer readings on the dial were noted. From the spring calibration chart the corresponding values of torque were found. The values of $f'$ were calculated for each r.p.m. and corresponding Re' was obtained from the calibration curve. From the knowledge of Re' values the apparent viscosity of the fluid was calculated. Following this procedure, viscosities of kerosine, several dilute suspensions of finely di-

vided powders of commercial kieselguhr in water, and iron catalysts in kerosine were determined. Some typical data are presented in Table I. On comparing the data for kerosine measured in the Ostwald viscometer and the present instrument, it will be observed that a deviation less than 2% was obtained.

The present instrument can be employed for the measurement of viscosity of a wide variety of materials such as muds, sand slurries, and fine coal suspensions in water. The working range of the apparatus can be extended to suit a particular fluid by use of a watch spring of proper stiffness. From the data given in Table I, it will be seen that there is a trend for the viscosity to increase with the Re'. Further work is needed to determine the causes for this behavior. A number of secondary effects such as centrifugal and end effects, however, may be expected to occur in an instrument of this type. Wilhelm et al. (6) observed that before the installation of the baffles in their viscometer, operation was unstable in the turbulent range with liquids of about 60 centipoises or less. Also, they obtained different torque vs.

### Table I. Typical Experimental Data

| R.P.M. | Deflection, Degrees | Torque, Gram Cm. | $f \times 10^5$ | Re | Viscosity, Cp. |
|---|---|---|---|---|---|
| Kerosine | | | | | |
| Density = 0.795 gram/cc. at 30.2° C. Viscosity by Ostwald pipet = 1.242 cp. | | | | | |
| 550 | 12 | 6.25 | 2.60 | 380 | 1.150 |
| 590 | 16 | 7.25 | 2.62 | 375 | 1.251 |
| 650 | 22 | 8.25 | 2.46 | 422 | 1.220 |
| 710 | 28 | 9.50 | 2.37 | 452 | 1.249 |
| 780 | 36 | 11.25 | 2.33 | 470 | 1.319 |
| 820 | 40 | 12.00 | 2.24 | 505 | 1.291 |
| 850 | 44 | 12.75 | 2.22 | 510 | 1.310 |
| 915 | 52 | 14.25 | 2.14 | 552 | 1.318 |
| | | | | Average | 1.263 |
| | | | | Deviation | +1.7% |
| Water-Kieselguhr Slurry | | | | | |
| [a] Material. Commercial kieselguhr powder (infusorial earth) unsieved. Concentration, 5 grams/100 cc. of slurry. Density, 1.021 grams/cc. at 30.0° C. | | | | | |
| 450 | 8 | 5.50 | 2.66 | 365 | 1.259 |
| 500 | 12 | 6.25 | 2.45 | 425 | 1.201 |
| 550 | 16 | 7.25 | 2.35 | 460 | 1.221 |
| 600 | 22 | 8.25 | 2.24 | 505 | 1.213 |
| 650 | 28 | 9.50 | 2.20 | 525 | 1.264 |
| 700 | 34 | 10.75 | 2.15 | 550 | 1.299 |
| 750 | 40 | 12.00 | 2.09 | 572 | 1.339 |
| 810 | 48 | 13.50 | 2.01 | 624 | 1.325 |
| 870 | 56 | 15.00 | 1.94 | 660 | 1.346 |
| 925 | 64 | 16.50 | 1.89 | 696 | 1.356 |
| | | | | Average | 1.282 |
| Kerosine-Catalyst Slurry | | | | | |
| [b] Material. Iron (100), copper (3), calcium oxide (10), kieselguhr (30). Concentration, 10 grams solid/100 cc. of slurry. Density, 0.861 gram/cc. at 30.2° C. Period of aging, 1 month | | | | | |
| 600 | 24 | 8.75 | 2.82 | 327 | 1.580 |
| 665 | 32 | 10.50 | 2.76 | 340 | 1.684 |
| 700 | 36 | 11.25 | 2.67 | 360 | 1.674 |
| 760 | 44 | 12.75 | 2.56 | 390 | 1.678 |
| 800 | 50 | 13.75 | 2.50 | 410 | 1.680 |
| 845 | 56 | 15.00 | 2.44 | 430 | 1.691 |
| 875 | 60 | 15.75 | 2.38 | 450 | 1.674 |
| 900 | 64 | 16.50 | 2.37 | 452 | 1.714 |
| 950 | 72 | 18.00 | 2.32 | 472 | 1.733 |
| | | | | Average | 1.679 |
| Kerosine-Catalyst Slurry | | | | | |
| [c] Material. Iron (100), copper (8), calcium oxide (5), kieselguhr (15). Concentration, 20 grams solid/100 cc. of slurry. Density, 0.943 gram/cc. at 30.0° C. Period of aging, 1 month | | | | | |
| 555 | 24 | 8.75 | 3.01 | 285 | 1.837 |
| 620 | 32 | 10.50 | 2.89 | 310 | 1.886 |
| 675 | 40 | 12.00 | 2.79 | 332 | 1.917 |
| 730 | 48 | 13.50 | 2.69 | 355 | 1.939 |
| 780 | 56 | 15.00 | 2.61 | 377 | 1.951 |
| 830 | 64 | 16.50 | 2.54 | 396 | 1.976 |
| 880 | 72 | 18.00 | 2.46 | 420 | 1.976 |
| 925 | 80 | 19.50 | 2.42 | 438 | 1.991 |
| | | | | Average | 1.934 |

[a] Displacement specific gravity, 2.280. Particle size, 40 microns, average microscopic.
[b] Displacement specific gravity, 2.310. Particle size, 80 microns, hindered sedimentation.
[c] Displacement specific gravity, 2.82. Particle size, 51 microns, hindered sedimentation.
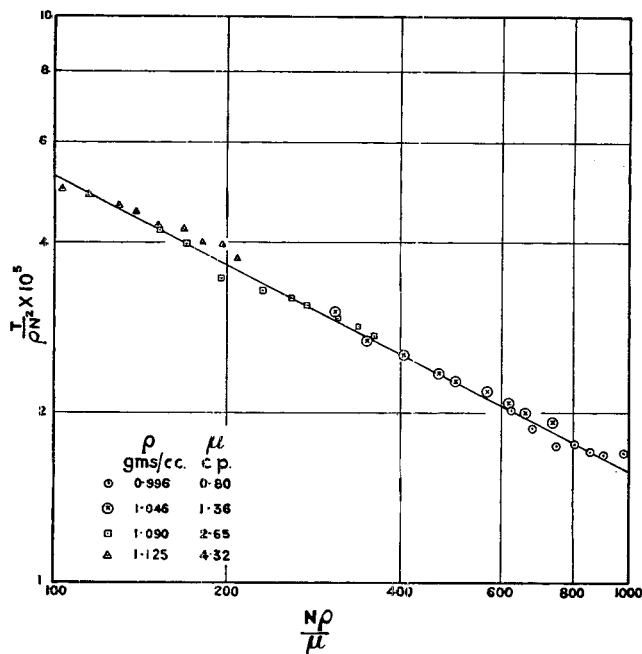
**Figure 4. Calibration curve for viscometer**

Replacement of the present mechanical arrangement by an electromagnetic system, in which the small torque on the cup would be measured by balancing it against the action of a coil carrying an electric current in a magnetic field, as in a large moving coil galvanometer (4).

A jacket around the outer casing with necessary fittings to serve as a constant temperature bath. This would enable viscosity determination at different temperatures.

### ACKNOWLEDGMENT

The authors wish to express their sincere thanks to S. R. Sen Gupta and G. H. von Hoessle for their kind interest and encouragement during the course of this investigation. They also wish to acknowledge the kind assistance received from A. K. Basu and the workshop staff of this institute in fabricating the instrument.

### NOMENCLATURE

$f$ = friction factor, dimensionless
$f'$ = specific friction factor
$h$ = height of inner cylinder, cm.
$N$ = number of revolutions per minute
$r_1$ = radius of inner cylinder, cm.
$r_2$ = radius of outer cylinder, cm.
$R$ = radius of pulley, cm.
Re = Reynolds number, dimensionless
Re' = specific Reynolds number
$T$ = torque, arbitrary units, gram-cm.
$W$ = applied load, grams
$w$ = equivalent of friction in rotating system, grams
$\eta$ = viscosity, poises
$\varphi$ = function
$\mu$ = viscosity, centipoises
$\pi$ = constant
$\rho$ = density, grams per cc.
$\Omega$ = angular velocity, radians per second

r.p.m. readings by changing the method of accelerating the rotor from rest to a high speed. It is important, therefore, to restrict the measurements within the range of $N$ in which such effects are maintained constant and the operation of the instrument is stable. In the present work, the speed of the rotor in the range of 500 to 900 r.p.m. has been found to give satisfactory results.

### PROPOSED REFINEMENTS

While working with this instrument it was found that certain modifications are possible which would make it more sensitive and easier to operate.

Replacement of the ordinary motor by a synchronous one. For the variation of the speed of the rotor, an r.p.m. regulator of the continuously variable speed transmission type may be used.

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# Use of 1,2-Naphthoquinone-4-sulfonate for the Estimation of Ethylenimine and Primary Amines

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Dilute aqueous solutions of ethylenimine or of $n$-butylamine react with 1,2-naphthoquinone-4-sulfonate at pH 10.3 to give reddish dyes which are extracted with chloroform. Quantitative estimations of the amines can be made by measuring absorbance at 420 and 450 m$\mu$, respectively. Ethanolamine also forms a reddish dye, but this product is not extracted from an aqueous solution with chloroform; extraction can be effected with isoamyl alcohol and quantitative determination carried out by measuring absorbance at 420 m$\mu$.

IN CONNECTION with the study of certain compounds related to ethylenimine, the authors discovered that dilute solutions of this imine give a rapid color reaction with potassium 1,2-naphthoquinone-4-sulfonate, a reagent used in Folin's test for amino acids (4, 7). Since the literature, as far as could be ascertained, contains no reference to any specific analytical method for ethylenimine, it seemed desirable to exploit the reaction as a means for estimating this increasingly important substance.

Whereas others, in the determination of amino acids, have used

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reducing agents (*1*, *4*, *10*) or acetone (*6*) to eliminate the color of the unreacted reagent, the present investigators found it possible to accomplish this by extracting the formed dye with an organic solvent, preferably chloroform; at the same time the sensitivity of the test was increased. The absorbance of the dye in the extraction solvent at 420 m$\mu$ is linear with ethylenimine concentration over the range studied. Ethanolamine, which may be found in aqueous ethylenimine solutions as a hydrolysis product, gives a color reaction similar to that of ethylenimine, but the dye so formed remains almost completely in the aqueous phase on extraction with chloroform.

The adaptability of the analytical method for ethylenimine to related substances was shown through the development of procedures for estimating ethanolamine and *n*-butylamine and by the results of qualitative tests with a number of amines and their derivatives. Whereas the dye formed from ethanolamine is almost unextractable from aqueous solution with chloroform, it is taken up readily by isoamyl alcohol. *n*-Butylamine, as does ethylenimine, forms a chloroform-extractable dye. The organic solutions of the dyes derived from ethanolamine and *n*-butylamine obey the Beer-Lambert law within the ranges of concentration studied. Qualitative tests indicate that ammonia forms a dye with the naphthoquinone sulfonate which is not chloroform-extractable, while quaternary ammonium compounds, tertiary amines (including *N*-alkylethylenimines), hindered secondary amines, and carboxylic amides do not react with the reagent.
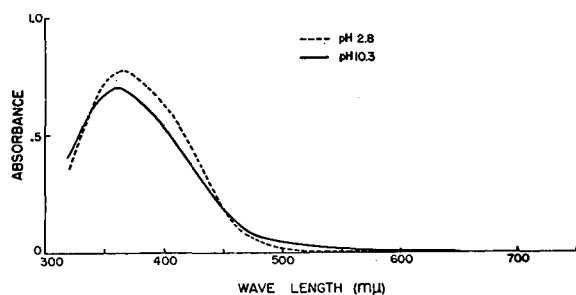


**Figure 1. Spectral curves for 3 × 10⁻⁴*M* quinone reagent in acid and base**

By the use of 1,2-naphthoquinone-4-sulfonates it should prove possible to determine the components of many amine mixtures by virtue of differences in the rates of color formation, in the absorption spectra of the dyes formed, or in the extractability of the latter. The application of the method presented herein to the estimation of trace quantities of amine atmospheric pollutants is recommended.

### PROPERTIES OF QUINONE REAGENT

The potassium salt of 1,2-naphthoquinone-4-sulfonic acid was used in the present work because it could be obtained easily in a pure state. Spectrophotometric examination of 3 × 10⁻⁴*M* aqueous solutions of this compound showed virtually identical spectra at pH 10.3 and 2.8 (Figure 1).

To determine the extractability of the naphthoquinone sulfonate by various solvents, 5-ml. portions of quinone reagent previously diluted to 3 × 10⁻⁴*M* were shaken with 2-ml. portions of solvent, and the color distribution was observed visually. Results showed that the quinone reagent was not extracted by the following solvents: chloroform, isoamyl alcohol, carbon tetrachloride, xylene, *n*-butyl alcohol, ethyl acetate, tributyl phosphate, trichlorobenzene, bromobenzene, and tri-*o*-cresyl phosphate.

On standing, the quinone reagent changed in color and absorption spectrum. This change was largely avoided by storage in a

cold, dark place; nevertheless, the reagent was freshly prepared each day to ensure best results.

### REACTIVITY AND EXTRACTABILITY

Spectral curves were obtained for 3 × 10⁻⁴*M* aqueous solutions of the quinone reagent to which excess quantities of certain amines had been added (Figures 2 and 3). For blanks, water containing only buffer was used. Although the final pH had no effect on the shape of the curves, reaction only took place under basic conditions; hence it is only the free base form of an amine that reacts. Schmidt (*8*) has used this fact in the estimation of sulfonamides which react with the quinone even in acid solution, as they do not form ammonium ions; amines do not interfere with the sulfonamide determination when the medium is acidic.
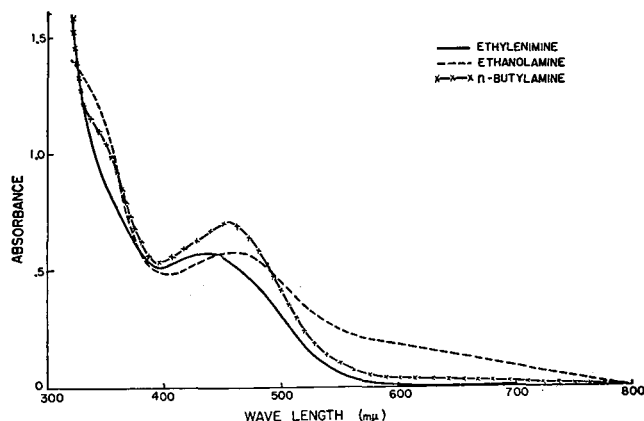


**Figure 2. Spectral curves for 3 × 10⁻⁴*M* quinone reagent reacted with excess ethylenimine, ethanolamine, or *n*-butylamine at pH 10.3**
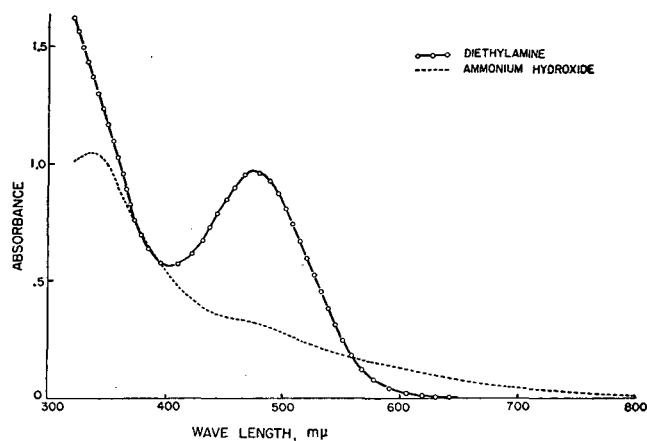


**Figure 3. Spectral curves for 3 × 10⁻⁴ *M* quinone reagent reacted with excess diethylamine or ammonium hydroxide at pH 10.3**

The reactivities of several amines and related compounds were observed qualitatively by adding up to a fivefold excess of amine to quinone reagent (3 × 10⁻⁴*M*) adjusted to pH 10.3. Noticeable color change from light yellow to orange or brown indicated that reaction had taken place. Generally, primary amines gave a more orange and secondary amines a browner color. Ethylenimine, *n*-butylamine, ethanolamine, diethylamine, *N*-butylethanolamine, diethanolamine, ammonium hy-

droxide, sulfanilamide, and aniline reacted; N-butylethyl-enimine, triethylamine, diisopropylamine, tetramethylammonium hydroxide, acetamide, formamide, dimethylformamide, and dimethylaniline showed no reaction. When 5-ml. portions of the preceding reaction mixtures were shaken with 2-ml. portions of solvent, it was observed visually that the dyes were extracted by solvent in some cases, partially in others, and not at all in still others (Table I).

**Table I. Extractability with Typical Solvents of Dyes Formed from Several Amines and Related Compounds**

| Compound | Chloroform[a] | Isoamyl Alcohol | Ethyl Acetate |
|---|---|---|---|
| Ethylenimine | + | + | + |
| N-Butylethanolamine | + | * | * |
| n-Butylamine | + | + | + |
| Ethanolamine | 0 | + | 0 |
| Diethylamine | + | * | * |
| Diethanolamine | 0 | * | * |
| Ammonium hydroxide | 0 | * | 0 |
| Sulfanilamide | 0 | + | * |
| Aniline | + | + | + |

[a] + extracted, * partially extracted, 0 not extracted.

Additional studies showed that the dye formed from ethanol-amine was not extracted by carbon tetrachloride, xylene, bromo-benzene, or 1,2,4-trichlorobenzene, and was partially extracted by n-butyl alcohol, tributyl phosphate, and tri-o-cresyl phosphate. All of these solvents extracted completely the dye formed from ethylenimine. The choice of chloroform as the solvent to be used in the analysis of ethylenimine was based on the ease of separation of phases; xylene and ethyl acetate formed emulsions which did not break readily.

## REAGENTS

**Potassium 1,2-Napthoquinone-4-sulfonate** was made according to the method of Martin and Fieser (2).

**Quinone Reagent** was freshly prepared each day by dissolving 0.138 gram of potassium 1,2-naphthoquinone-4-sulfonate in 100 ml. of distilled water.

**N-Butylethylenimine** was synthesized according to the general procedure given by Shirley (9) for ethylenimine and had physical constants in excellent agreement with those reported by Elderfield (3). It was stored over potassium hydroxide pellets.

**Other Reagents.** Ethylenimine, as purchased, was stored over potassium hydroxide pellets; n-butylamine was redistilled from potassium hydroxide. Other chemicals were used as purchased. Distilled water was used in all experiments, and the pH was adjusted where necessary with 0.05M phosphate buffer of pH 11.7 or with Clark and Lubs phthalate buffer (5) of pH 2.2.

## APPARATUS

Spectrophotometric determinations were made on a Beckman Model DU spectrophotometer with 1-cm. quartz cells. Measurements of pH were made with a Beckman Model G pH meter.

## PROCEDURES FOR QUANTITATIVE ANALYSIS

**Ethylenimine.** A 50-ml. portion of an aqueous ethylenimine solution is mixed with 10 ml. of quinone reagent and 1 ml. of phosphate buffer in a glass-stoppered 125-ml. Erlenmeyer flask. After a reaction period of 1 minute, 10 ml. of chloroform are added, along with a Teflon-covered magnetic stirring bar; the flask is stoppered and its contents are stirred vigorously for 5 minutes. After the phases have separated, the chloroform solution is pipetted off and transferred to the spectrophotometer; the absorbance is read against a chloroform blank at 420 mμ. From solutions of ethylenimine at various known concentrations, a calibration curve is constructed, from which the concentration of unknown solutions may be read.

**n-Butylamine.** The procedure for n-butylamine is similar to that for ethylenimine, except that the reaction period is 20 minutes and the absorbance is read at 450 mμ.

**Ethanolamine.** A 50-ml. portion of an aqueous ethanolamine solution is mixed with 10 ml. of quinone reagent and 1 ml. of
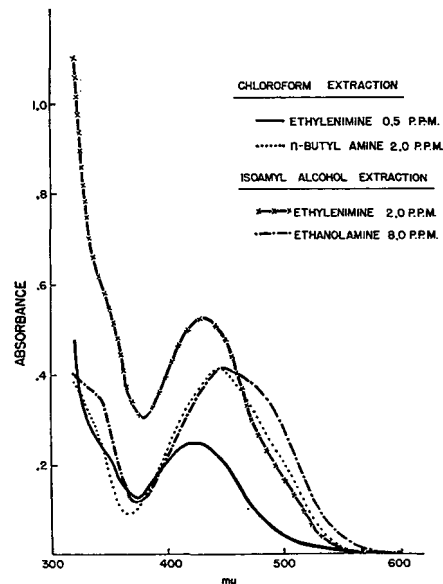


Figure 4. Spectrophotometric curves of extracted reaction products of potassium 1,2,-naphthoquinone-4-sulfonate

phosphate buffer in a glass-stoppered Erlenmeyer flask. After a reaction period of 20 minutes, 15 ml. of isoamyl alcohol are added, along with a Teflon-covered magnetic stirring bar; the flask is stoppered and the mixture is stirred vigorously for 5 minutes. After the phases have separated, the isoamyl alcohol layer is decanted and centrifuged to clarify it. The absorbance is read at 450 mμ. A calibration curve is constructed as in the previous procedures.

## RESULTS AND DISCUSSION

**Spectral Curves of Extracted Dyes.** Absorption spectra of the chloroform extracts of ethylenimine and n-butylamine and of the isoamyl alcohol extracts of ethylenimine and ethanolamine (both by the ethanolamine procedure) are shown in Figure 4.

**Reaction Rates.** The effect of varying the reaction period in the quantitative procedures was investigated (Figure 5). The variations in absorbance are evidently due to the different reaction rates of the three amines. Ethylenimine reacts rapidly, but ethanolamine and n-butylamine must stand for a long period with the quinone reagent before reaction is complete. However, it was found that 20 minutes is sufficient to give reproducible results in the case of the latter two amines and to ensure that
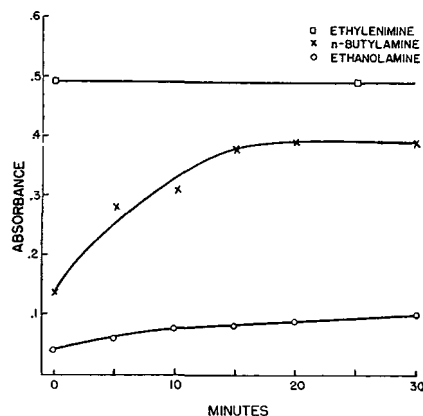


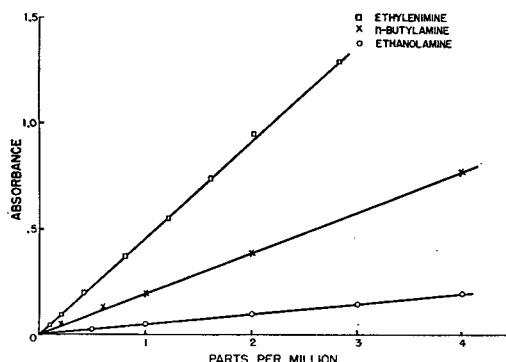Figure 5. Effect of holding time on absorbance

Figure 6.   Sensitivity curves for amine
determinations

**Table II.   Degree of Interference of Ethanolamine in Ethylenimine Test[a]**

| Solution | Concn., P.P.M. | Absorbance at 420 m$\mu$ (Av.) |
|---|---|---|
| Reagent blank | | 0.015 |
| Ethylenimine | 1 | 0.491 |
| Ethanolamine | 6 | 0.025 |
| Ethanolamine | 10 | 0.048 |
| Ethanolamine | 24 | 0.062 |
| Ethylenimine-ethanolamine | 1 to 6 | 0.521 |
| Ethylenimine-ethanolamine | 1 to 10 | 0.541 |
| Ethylenimine-ethanolamine | 1 to 24 | 0.546 |

[a] Chloroform extraction method.

small errors in timing would not introduce appreciable errors in the absorbance readings.

**Sensitivity and Adherence to the Beer-Lambert Law.** The sensitivities of the analyses are 0.46 for ethylenimine, 0.19 for *n*-butylamine, and 0.048 for ethanolamine, where sensitivity is defined as

$$\frac{\text{Absorbance of sample} - \text{absorbance of reagent blank}}{\text{parts per million of amine}}$$

The determinations show excellent adherence to the Beer-Lambert law over the measurable concentration ranges (Figure 6) as well as great sensitivity. Colorimeter readings were also made, with the Klett-Summerson photoelectric colorimeter, with No. 42 or No. 44 filters. These readings were likewise linear with concentration, and the measurable concentration ranges were about the same as with the spectrophotometer.

**Degree of Interference of Ethanolamine in the Ethylenimine Test.** The presence of a relatively high concentration of ethanolamine in an aqueous solution containing ethylenimine has but slight effect on the determination of the latter by the chloroform extraction method. Table II shows that the sensitivity of this method for ethanolamine is but 1/100th that of the test for ethylenimine.

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# Colorimetric Determination of the Perchlorate Ion

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The perchlorate ion gives a precipitate with the cupric tetrapyridine cation. The ensuing decrease in the color intensity of solutions of cupric tetrapyridine ion can be measured spectrophotometrically and used for the quantitative determination of the perchlorate ion. The method has also been applied to the determination of the perchlorate ion in organic perchlorates.

CUPRIC tetrapyridine perchlorate, studied and characterized by Weinland, Effinger, and Beck (*2*), had been suggested by Shead and Bailey (*1*) as a means of identifying the perchlorate ion. The object of the present investigation was to use the cupric tetrapyridine complex for the quantitative estimation of the same ion. The solubility of the cupric tetrapyridine perchlorate in water, although slight, excludes a gravimetric method. However, the solutions of salts of the cupric tetrapyridine complex in aqueous pyridine show an intense blue color, the intensity of which decreases as part of the complex is precipitated as per-

chlorate. This fact makes the colorimetric determination of perchlorate possible.

## INORGANIC PERCHLORATES

Using the conditions described by Shead and Bailey (*1*) a solution of cupric tetrapyridine nitrate in a mixture of water and pyridine was prepared; varying quantities of ammonium perchlorate were added to equal volumes of this solution and after crystallization of the cupric tetrapyridine perchlorate, the absorbance of the solutions was measured. A curve was thus obtained, showing the absorbance of a solution of the complex as a function of the amount of perchlorate added. The results obtained with this curve, however, were satisfactory only as long as no ions other than perchlorate and nitrate were present. Quantities of chlorate, chloride, or sulfate, of the same order of magnitude as the amount of perchlorate present, affected the color or gave precipitates. As it is often necessary to determine the perchlorate in the presence of these interfering ions, conditions were sought under which their influence was suppressed.

The following four points were studied.

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Table I.   Influence of Various Salts on Absorbance of Solutions of Cupric Tetrapyridine Nitrate

| | | Na₂SO₄ | | KClO₃ | | KNO₃, | NH₄Cl, | MgCl₂, | CaCl₂, | BaCl₂, |
|---|---|---|---|---|---|---|---|---|---|---|
| | None | .8 | .20 | .8 | 20 | 20 | 20 | 20 | 20 | 20 |
| Absorbance of solutions | | | | | | | | | | |
| Containing 0.2 mg./ml. of perchlorate | 0.675 | 0.667 | 0.640 | ... | 0.642 | 0.672 | 0.682 | 0.681 | ... | ... |
| Containing 0.4 mg./ml. of perchlorate | 0.481 | ... | 0.498 | 0.498 | 0.501 | 0.516 | 0.508 | 0.501 | 0.511 | 0.521 |

Table II.   Influence of Temperature on Absorbance of
Cupric Tetrapyridine Nitrate Solution

| Temp., °C. | 11 | 21 | 25 | 30 | 35 | 40 | 45 | 50 |
|---|---|---|---|---|---|---|---|---|
| Absorbance | 0.272 | 0.267 | 0.265 | 0.255 | 0.245 | 0.232 | 0.222 | 0.175 |

**Effect of Pyridine Concentration.** The addition of more pyridine to the solution prevented the formation of precipitates by sulfates or chlorides, and deepened the color of the cupric tetrapyridine complex. Figure 1 shows the variation of the absorbance of a solution of the complex as a function of the quantity of pyridine added. At equal volumes of water and pyridine, the maximum absorbance of the complex is practically reached. Further additions of pyridine increase the solubility of the cupric tetrapyridine perchlorate and must, therefore, be avoided.

**Effect of Various Anions on Color of Complex.** The absorbance of solutions containing varying quantities of sodium chloride, sodium sulfate, potassium bromide, or potassium nitrate was measured (Figure 2). Sodium chloride solutions give the deepest color with the cupric tetrapyridine complex, bromide has a smaller influence, and sulfate and nitrate do not change the color at all. Moreover, in the presence of a high concentration of sodium chloride the effect of other anions (chlorates, phosphates, nitrates, bromides, etc.) on the color of the solution was almost entirely suppressed (Tables I and III).

**Effect of Various Cations.** The presence of sodium, potassium, ammonium ions, or of alkaline earth metals in concentrations up to 10 mg. per ml. has no influence. Higher concentrations affect the extinction slightly (Table I). Other metals (silver, manganese, iron, aluminum, zinc), on the other hand, were found to form either complexes with pyridine or precipitates of the hydroxides; they have, therefore, to be removed before determination of the perchlorate.

**Effect of Temperature.** The effect of changes of temperature on the color of the solution is shown in Table II.

In the range between 10° and 25° C. there is very little change in the absorbance of the solution.

**PROCEDURE**

As a result of these studies the following method was adopted. Three stock solutions are required.

Solution A.   A solution prepared by dissolving about 25 grams of cupric nitrate trihydrate in water, adding 540 ml. of pyridine, and making up with water to 1 liter. (Kept in a brown bottle, this solution did not change its optical properties for at least 2 years.)

Solution B.   An aqueous solution of 20 grams of sodium chloride per 100 ml.

Solution C.   A solution containing 58.75 mg. of ammonium perchlorate per ml. (50 mg. of perchlorate anion per ml.).

**Preparation of Calibration Curve.** A dozen solutions are prepared containing: 2 ml. of solution A; 20 ml. of solution B; varying quantities of solution C (increasing gradually the perchlorate content in the final solutions from zero to 1.6 mg. per ml.); and water to 25 ml.

These solutions are left for 48 hours to crystallize (for concentrations up to 0.5 mg. per ml. 24 hours are sufficient). The solutions are filtered and the absorbances of the filtrates are measured in a Beckman DU spectrophotometer at 6350 A. The results are plotted against the concentrations of perchlorate ion, as shown in Figure 3.

**Analysis of Sample.** A solution is prepared from the sample as described in the preparation of the calibration curve (2 ml. of solution A, 20 ml. of solution B, a weighed quantity of the unknown sample, and water to 25 ml.). After 48 hours, the absorbance of the supernatant solution is measured, and the concentration of perchlorate is read from the calibration curve. (For technical routine analysis, results can be obtained after a few hours if the solution is shaken mechanically. The calibration curve has then to be prepared under the changed conditions. The accuracy of the method will be reduced, but will still suffice in many cases.)

**RESULTS**

In Table III results are given of determinations carried out both in the absence and in the presence of other salts. The accuracy of the method is within 0.01 mg. per ml. At concentrations of 0.5 to 1.2 mg. of perchlorate per ml., the error lies between 1 and 2%.

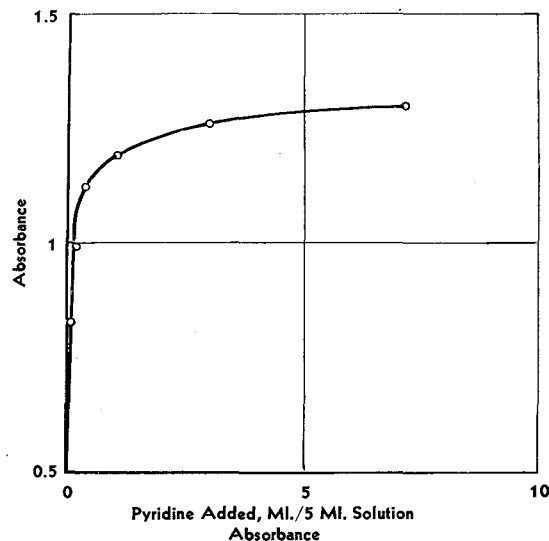If the concentration of other ions in the solution rises above 10



Figure 1.   Change in color intensity of cupric tetrapyridine nitrate solution on addition of pyridine
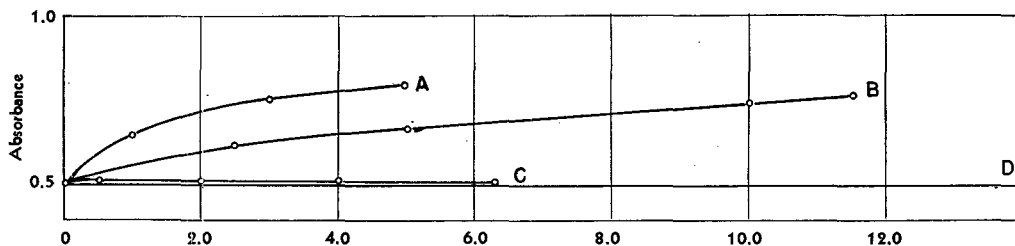


Figure 2.   Change in color intensity of cupric tetrapyridine nitrate solution on addition of anions

A.   NaCl added               C.   Na₂SO₄ added
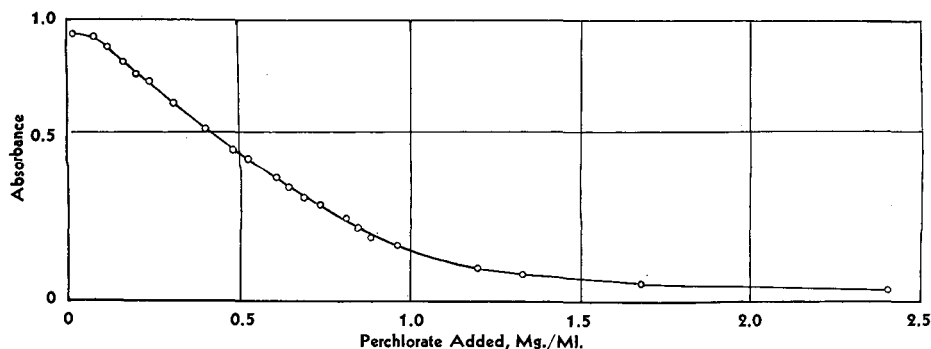B.   KBr added                D.   KNO₃ added
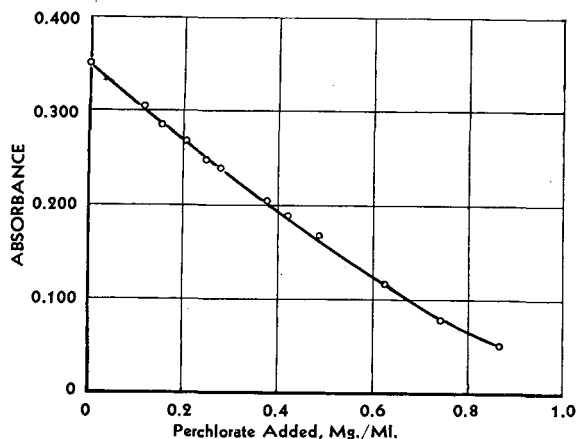
**Figure 3. Calibration curve for inorganic perchlorates**



**Figure 4. Calibration curve for organic perchlorates**

or more, which is not sufficient for organic analyses. For solutions saturated with sodium chloride the calibration curve is practically a straight line for much lower concentrations. It is thus possible to determine quantities of perchlorate even below 100 $\gamma$ per ml. with an accuracy within ±5 $\gamma$.

**PROCEDURE**

**Preparation of Calibration Curve.** Solutions of potassium perchlorate are prepared containing 0.0, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, and 50.0 mg. of perchlorate ion, respectively, 2 ml. of stock solution A, and water to 25 ml. These solutions are saturated with an excess of dry sodium chloride of analytical grade. They are then shaken vigorously, left to crystallize overnight, and filtered through dry filter paper. The absorbance of the filtrates is measured in a Klett-Summerson colorimeter with filter 66 (transmittance range from 6400 to 7000 A.), and is plotted against the concentration of the perchlorate ion (Figure 4).

**Analysis of Sample.** The sample is dissolved in water to a concentration of between 50 and 600 $\gamma$ of perchlorate ion per ml. A volume of 2 ml. of stock solution $A$ is added, and the solution is made up to 25 ml. If only very small quantities of sample are available, this volume can be correspondingly smaller, the only limit being that required for the colorimetric measurement. This solution is shaken with an excess of dry sodium chloride, kept overnight, and filtered. The absorbance of the filtrate is measured and the perchlorate ion content is read from the calibration curve.

**RESULTS**

The percentage of perchlorate ion contained in the organic perchlorate is readily obtained from the perchlorate content of the solution. The molecular weight of the organic compound can be derived from this determination. Table IV shows results obtained by this method.

mg. per ml., in some cases slight deviations occur which, however, do not cause an error greater than 0.03 mg. per ml. If very high concentrations of another ion are present, it is advisable to prepare a calibration curve corresponding to these conditions.

## ORGANIC PERCHLORATES

Owing to their low solubility in water, organic perchlorates are often used for the separation and purification of organic bases. While the combustion analysis of these perchlorates is not practical, because of their tendency to explosive decomposition, the colorimetric method described above makes it possible to determine the perchlorate ion directly, even in very dilute solutions of the salts.

Under the conditions described, optimal accuracy is obtained only with quantities of about 0.12 mg. of perchlorate ion per ml.

**LITERATURE CITED**

(1) Shead, A. C., and Bailey, P. S., *Mikrochemie*, **33**, 1 (1947).
(2) Weinland, R., Effinger, K., and Beck, V., *Arch. Pharm.*, **265**, 352 (1927).

**Table III. Results Obtained Using Calibration Curve (Figure 3)**

| | Salt Present Besides NaCl, Mg./Ml. | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | KBr, 8 | None | Na₂SO₄, 8 | KClO₃, 8 | KNO₃, 8 | NH₄Cl, 8 | BaCl₂, 8 | CaCl₂, 8 | MgCl₂, 8 | KBr, 8 | None |
| **Perchlorate, mg./ml.** | | | | | | | | | | |
| Added | 0.48 | 0.52 | 0.56 | 0.60 | 0.68 | 0.72 | 0.76 | 0.84 | 0.88 | 0.92 | 1.00 |
| Found | 0.47 | 0.52 | 0.57 | 0.60 | 0.69 | 0.73 | 0.76 | 0.85 | 0.89 | 0.90 | 0.98 |

**Table IV. Determination of Perchlorate Ion in Various Organic Perchlorates**

| Substance | % of Perchlorate Ion | | Molecular Weight | |
| --- | --- | --- | --- | --- |
| | Found | Theoretical | Found | Theoretical |
| Benzoylcholine perchlorate | 32.2, 32.4, 31.9, 32.4, 32.3 | 32.4 | 308, 307, 312, 307, 308 | 307 |
| Brucine perchlorate | 19.7, 20.3 | 20.1 | 502, 490 | 495 |
| Quinaldine perchlorate | 42.4, 41.5, 42.0 | 40.4 | 236, 240, 237 | 243.7 |
| Trimethylbutylammonium perchlorate | 45.8, 46.6 | 46.2 | 217, 214 | 215.5 |
| Trimethylheptylammonium perchlorate | 39.3, 38.1, 38.6 | 38.8 | 254, 262, 258 | 257.5 |
| Trimethyloctylammonium perchlorate | 36.1, 37.4, 37.3 | 36.6 | 276, 266, 267 | 271.5 |
| Trimethyldodecylammonium perchlorate | 30.9, 30.6 | 30.4 | 322, 325 | 328 |

# Amperometric Instrument for Quantitative Determination of Oxygen Dissolved in Oil-Field Brines

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The design and operation of portable amperometric instruments for the rapid quantitative determination of dissolved oxygen in brine have been investigated, and a more simple, accurate, and reliable instrument than those previously described in the technical literature has been developed. An analysis of the effects of temperature and oxygen content of brine upon the diffusion current of rotating platinum microelectrodes has made possible the simplified calibration of such instruments. This paper describes in detail the design principles of an instrument for determining the dissolved-oxygen content of oil-field brines. Operation and calibration of the instrument are discussed, and the advantages and disadvantages of different electrode designs are pointed out. It is concluded that the amperometric instrument will yield accurate reproducible results when properly applied.

ONE of the problems encountered in field studies of the corrosivity of oil-field brines by Bureau of Mines engineers (14) is the difficulty of making reliable quantitative determination of dissolved oxygen in the brines (13). Based on the amperometric technique of Kolthoff (3) and others, Marsh (7) developed a portable amperometric instrument specifically for determining dissolved oxygen in oil-field brine. Before this, one of several modifications of the method developed by Winkler (15) was used. The modified Winkler methods are sensitive and relatively simple; but they have the disadvantage, particularly when used in the analysis of oil-field brines, of being subject to interference from iron, calcium, magnesium, reducing agents, and other substances present in many brines.

Another disadvantage of chemical methods for field use is the inconvenience of transporting the necessary apparatus and reagents as compared with the portability of the amperometric instrument. The portability of the equipment is an important factor in accurate determination of the dissolved-oxygen content of oil-field brines, as it is difficult to avoid a change in the oxygen content of the sample if it must be transported to a laboratory. This source of error is most serious with samples having a low oxygen content. The portability of the amperometric instrument permits the analyst to make the determination at field sampling points.

In addition, the amperometric instrument is not affected by certain salts, commonly found in oil-field brines, which interfere with the Winkler method. Marsh (7) reported the results of tests on samples of water using an amperometric meter of his design. The water samples had varying pH values and contained ferrous and ferric salts, sulfides, and various organic compounds. Interference with the Winkler method by many ions makes direct comparison of the two methods impossible. However, mixing a brine that does not contain interfering ions and the oxygen content of which can be accurately determined by the modified Winkler method with a deaerated brine containing the interfering ions gives a satisfactory indirect comparison. Free chlorine interferes with the amperometric method, but the results may be corrected if the concentration of free chlorine is known. The chief disadvantage of the amperometric method is that it cannot be used unless the aqueous solution is a good electrical conductor. Erratic results are obtained with brines having a resistivity greater than about 2 ohm-meters. This resistivity is roughly equivalent to that of a brine containing 3000 p.p.m. of sodium chloride at room temperature. Some work has been done with the addition of salt to water samples before measurement, but results of that work are inconclusive.

## Table I. Dissolved-Oxygen Meter Calibration Data

| Temp., ° F. | Diffusion Current, μa. | Dissolved Oxygen, P.P.M. | | Difference | |
|---|---|---|---|---|---|
| | | By D.O. meter | By Winkler | P.P.M. | % |
| 40.8 | 6.0 | 0.62 | 0.65 | −0.03 | − 4.6 |
| 40.7 | 26.5 | 2.73 | 2.60 | +0.13 | + 5.0 |
| 41.0 | 35.0 | 3.57 | 3.55 | +0.02 | + 0.6 |
| 41.0 | 56.0 | 5.72 | 5.70 | +0.02 | + 0.4 |
| 67.3 | 43.5 | 2.79 | 2.75 | +0.04 | + 1.5 |
| 67.7 | 50.0 | 3.19 | 3.30 | −0.11 | − 3.3 |
| 67.5 | 68.0 | 4.35 | 4.40 | −0.05 | − 1.1 |
| 67.3 | 70.0 | 4.49 | 4.50 | −0.01 | − 0.2 |
| 74.0 | 9.0 | 0.53 | 0.55 | −0.02 | − 3.6 |
| 74.0 | 32.0 | 1.88 | 1.87 | +0.01 | + 0.5 |
| 74.0 | 56.0 | 3.28 | 3.20 | +0.08 | + 2.5 |
| 74.0 | 66.0 | 3.87 | 3.75 | +0.12 | + 3.2 |
| 74.0 | 80.3 | 4.71 | 4.70 | +0.01 | + 0.2 |
| 74.0 | 100.0 | 5.87 | 5.90 | −0.03 | − 0.5 |
| 74.0 | 103.0 | 6.04 | 6.05 | −0.01 | − 0.2 |
| 76.3 | 23.0 | 1.31 | 1.35 | −0.04 | − 3.0 |
| 76.3 | 30.0 | 1.71 | 1.70 | +0.01 | + 0.6 |
| 76.3 | 39.5 | 2.25 | 2.35 | −0.10 | − 4.3 |
| 79.5 | 72.0 | 3.94 | 4.00 | −0.06 | − 1.5 |
| 87.0 | 22.0 | 1.10 | 1.05 | +0.05 | + 4.8 |
| 87.0 | 80.0 | 4.02 | 3.95 | +0.07 | + 1.8 |
| 86.8 | 111.0 | 5.59 | 5.55 | +0.04 | + 0.7 |
| 92.0 | 106.0 | 5.05 | 5.00 | +0.05 | + 1.0 |
| 96.8 | 32.0 | 1.45 | 1.30 | +0.15 | +11.5 |
| 96.8 | 80.0 | 3.62 | 3.25 | +0.37 | +11.4 |
| 96.5 | 124.0 | 5.64 | 5.00 | +0.64 | +12.8 |

Over-all accuracy of the instrument using the Winkler method as a standard with 5% brine for 69 determinations averaged 3.4%. Table I shows the data for the first 26 of these determinations. It may be seen that the average deviation from the Winkler determination is less than 0.1 p.p.m. of oxygen. Using the 69 values obtained, percentage error varies from about 5.6 at less than 1 p.p.m. of oxygen to 2.7 with more than 5 p.p.m. of oxygen. However, error in terms of oxygen content ranges from less than 0.05 p.p.m. with less than 1 p.p.m. of total oxygen to 0.14 at ranges above 5 p.p.m.

## DESCRIPTION OF INSTRUMENT

The basic amperometric instrument described in this paper offers a number of refinements over that developed by Marsh. It consists of a simple electrolysis H-cell, with the usual saturated calomel reference electrode and agar–potassium chloride bridge,
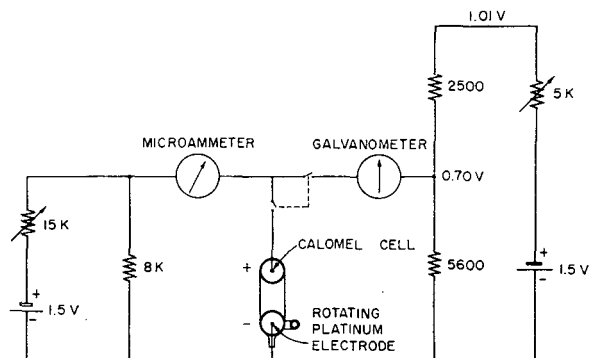


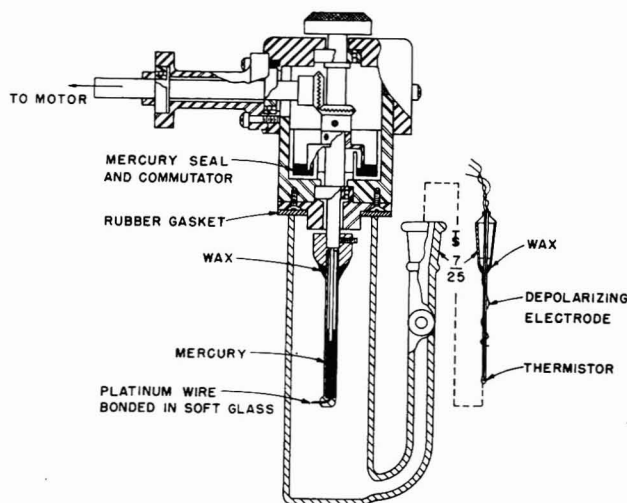**Figure 1. Simplified diagram of dissolved-oxygen meter**

**Figure 2. Motor, gear, and electrode assembly of dissolved-oxygen meter**

a rotating platinum microelectrode, a voltage source, and a suitable microammeter for measuring oxygen diffusion current. In addition, a means of determining the temperature of the brine sample is incorporated in the instrument. The basic diagram is shown in Figure 1.

A 24-volt direct current, geared-head, constant-speed motor (Delco 5069625), obtained as war surplus equipment, is used to rotate the platinum electrode. The output shaft speed of the motor is 150 r.p.m., which requires the use of 1 to 4 increase ratio to obtain the desired electrode speed of 600 r.p.m. The governor on the motor operates by making and breaking the armature circuit. Excellent results are obtained thereby, as indicated by the less than 2% change in speed with voltage inputs from 14 to 28 volts and less than 1% change at normal operating voltages. The current drain is less than 200 ma., and satisfactory operation is obtained from four 6-volt, filament supply-type batteries (Burgess F4BP) connected in series.

The motor, gear, and electrode assembly of the dissolved-oxygen meter are illustrated in Figure 2. The electrode is driven through beveled gears to allow entire enclosure of the motor within the instrument case. A mercury-cup commutator establishes connection with the platinum electrode in a manner similar to that described by Kolthoff (3). A much simpler arrangement, such as a wire extending through a hollow shaft and contacting the mercury head in the electrode directly, probably would be equally satisfactory.

The speed of rotation of the microelectrode is important, in that it affects the diffusion current. Although no attempt was made in the Bureau of Mines laboratory to find a definite relationship between speed of rotation and diffusion current, investigations by Kolthoff and Lingane (6), Roller (9), and Van Name and Edgar (12) show such a relationship. These authors found that diffusion current increases approximately as the 0.6th to the 0.85th power of the speed of rotation. Rosenthal and others (10) note that a rotational speed can be reached beyond which a further increase of speed has no effect on diffusion current. This is at variance with the reports of King and Braverman (2) concerning the rate of solution vs. velocity, the mechanics of which are similar, and it is believed that Rosenthal may have experienced cavitational effects at high speeds that would introduce observational errors. Most investigators have used a rotational speed of 600 r.p.m. with satisfactory results.

The platinum microelectrode is one of the most critical units of the instrument. Many types of electrodes were constructed and tested, but none was completely satisfactory. The most successful electrode tested was the glass-sealed type, with mercury contact to the platinum wire as shown in Figure 2. The exposed length of the platinum electrode is $1/8$ inch and its diameter is 0.032 inch. The development of fine cracks in the glass seal, almost invisible to the naked eye and usually first evidenced by erratic operation of the meter, was the primary cause of failure of such electrodes. Remedial measures for these cracks are not apparent, as careful annealing seemed to have little effect. Cleaning of the electrode with steel wool between determinations was thought at first to have caused cracking. However, elimination of the need for this cleaning by use of a third, depolarizing electrode did not prolong the life of the electrode. Marsh (7) used a brass rod coated with vinyl paint to support the micro-

electrode. The writers found that the coating on such electrodes tends to separate from the metal at the base of the platinum wire, exposing the brass and thus causing spurious readings. Experiments with a petroleum-wax coating, using an electrode similar to the ones used by Kolthoff (3) and Marsh (7), showed promise.

A −0.70-volt potential is applied to the rotating microelectrode. Operation at this voltage was chosen to provide maximum sensitivity and also to eliminate interference from ions having reduction potentials slightly more negative than −0.70 volt. This voltage is near the upper end of the first oxygen wave, as shown in Figure 3 (4).

The applied voltage must be measured directly at the electrode, because the microammeter used to measure the diffusion current has an appreciable resistance (3400 ohms). The measurement was made with a vacuum-tube voltmeter in the portable dissolved-oxygen meter of Marsh (7). However, portable vacuum-tube voltmeters are fragile, and their calibration tends to change with time. A simple potentiometric voltage-measurement circuit was used in this instrument to provide better reliability. The circuit consists of an inexpensive Weston standard cell (Muirhead Type D-440-A), a portable panel-mounting galvanometer, and a voltage divider. A 1.5-volt battery (Burgess 4FH) is used as the working cell. The galvanometer is used in initial standardization of the working cell voltage, in adjustment of the voltage applied to the rotating electrode, and in balancing the thermistor bridge for temperature measurement.

A saturated calomel cell is used as a reference electrode, in conjunction with the saturated salt bridge and rotating electrode, as shown in Figure 4. The cell is constructed in an inner container with a hole in the side for solution contact with the salt bridge. This construction facilitates renewal of the cell. A large mercury-to-calomel surface area is necessary to obtain the low internal resistance desirable when large reduction currents (100 to 200 μa.) are required. In the instrument designed by the writers, 3.5 sq. cm. of mercury surface is exposed.
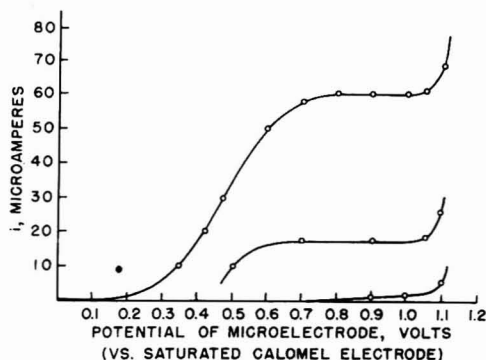


**Figure 3. Current-voltage curves of oxygen with rotating platinum microelectrode (4)**
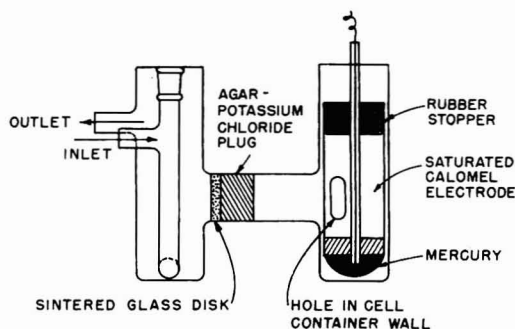


**Figure 4. Detail of H-cell used in dissolved-oxygen meter**

The sensing element used for measuring temperatures with the instrument is a small, sealed-in-glass thermistor (Western Electric Type 14B) inserted into the inlet line of the electrolysis cell, as shown in Figure 2. The resistance of the thermistor is determined with a Wheatstone bridge having a measuring arm calibrated directly in degrees Fahrenheit. There are two advantages in the

use of the thermistor for temperature determination: (1) The time that the thermistor requires to reach the true brine temperature is short enough (about 1 second) to allow almost instantaneous readings, and (2) a well-marked dial is much more convenient to read than the mercury column of a thermometer inserted in the cell.



**Figure 5. Schematic diagram of dissolved-oxygen meter**

Each operation of the instrument, such as calibration, temperature measurement, and diffusion-current measurement, involves throwing one lever switch, pressing the galvanometer switch, and turning a potentiometer knob to a null reading on the galvanometer. The switching arrangement used for connecting the various elem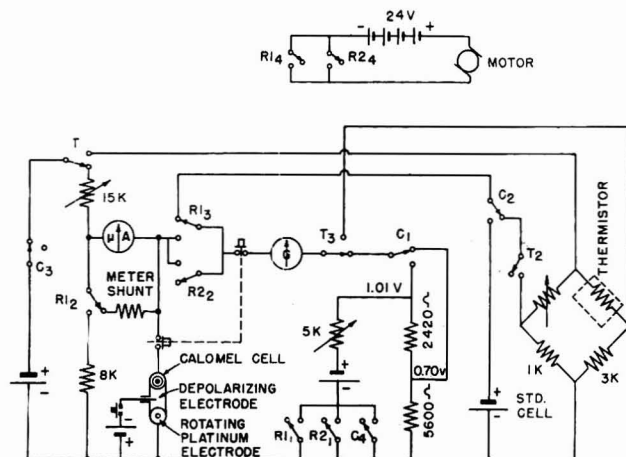ents of the instruments in the desired sequence is shown in the schematic diagram in Figure 5. The lever switches are a four-pole, two-position type commonly used in the construction of communication equipment. The instrument is wired so that the equipment will not be damaged if two or more switches are thrown inadvertently at the same time. A plate in the lid of the instrument prevents closing of the lid when any switch is in the operating position, eliminating the possibility of discharging the batteries unnecessarily. The galvanometer push button switch is a double-pole unit, with one pole used to connect the calomel cell into the circuit. This switch arrangement limits the time during which current is flowing in the electrolysis cell and thereby minimizes undesirable polarization effects of the rotating platinum microelectrode.
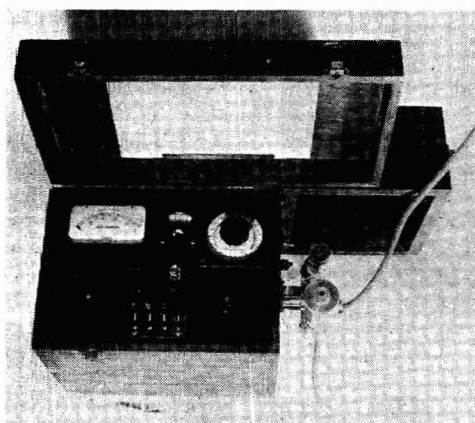


**Figure 6. Amperometric dissolved-oxygen meter in operating position**

A second push button switch on the side of the instrument is used to apply a depolarizing potential to the rotating electrode through a third electrode in the cell. This depolarizing electrode is a small platinum wire attached to the thermistor unit. Before each oxygen determination is made, a positive potential of 1.5 volts is applied once or twice for an interval of 10 to 20 seconds, or until the application of the potential does not further increase
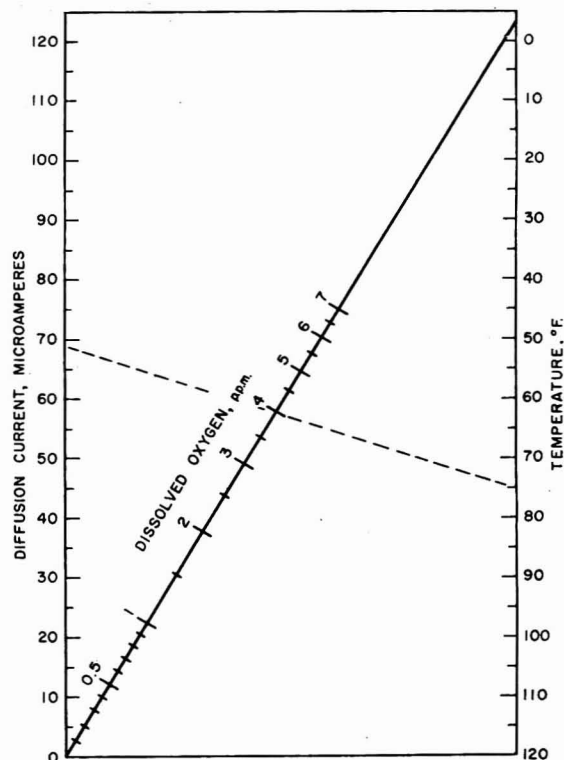


**Figure 7. Typical nomograph for use with dissolved-oxygen meter**

the equilibrium diffusion current. Use of a depolarizing electrode eliminates the necessity for constantly scraping the electrode. Figure 6 is a photograph of the instrument.

### CALIBRATION

A comprehensive investigation was made of the rotating microelectrode and the diffusion current resulting from reduction of oxygen dissolved in brine. The diffusion current of the electrode was calibrated in terms of oxygen concentration. In all calibration work, the modified Winkler method (13) was employed as the reference standard, and the brine was free of substances that interfered with the Winkler method.

The calibrations were made with synthetic brines prepared with tap water and table salt. A concentration of 50,000 p.p.m. of sodium chloride was used in all tests except those made to determine the effects of brine concentration on diffusion current. The brine was filtered before it was used to remove magnesium carbonate found in the commercial salt and then was stored in 5-gallon bottles. These bottles were immersed in a large, insulated water bath to minimize temperature changes. Nitrogen was bubbled through the brine to reduce the oxygen content to the desired concentration. Approximately 150 minutes were required to reduce the dissolved-oxygen content from about 8 to 0.2 p.p.m., although the concentration was reduced to 4 p.p.m. in about 20 minutes.

Two basic types of microelectrode were investigated—the bright platinum electrode and the platinized platinum electrode. The construction of both types was similar; and, with either one, glass-sealed units gave the most reproducible results. The platinized platinum electrode eliminates the need for frequent electrode depolarization. It is undoubtedly the more desirable electrode for use with pure solutions in laboratory work but is too easily poisoned or contaminated for use in the field. Surprisingly enough, little or no difficulty was experienced with flaking of platinum from the electrode or in the washing off of the platinized surface at the rotational speed used. As this instrument was
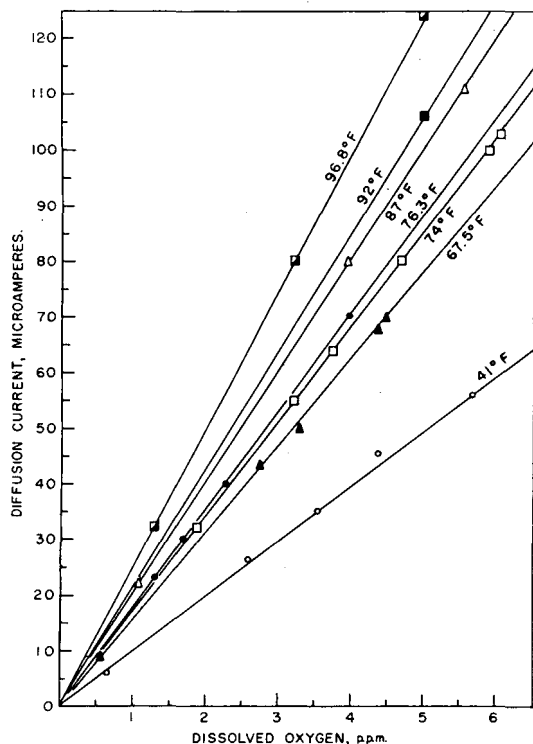
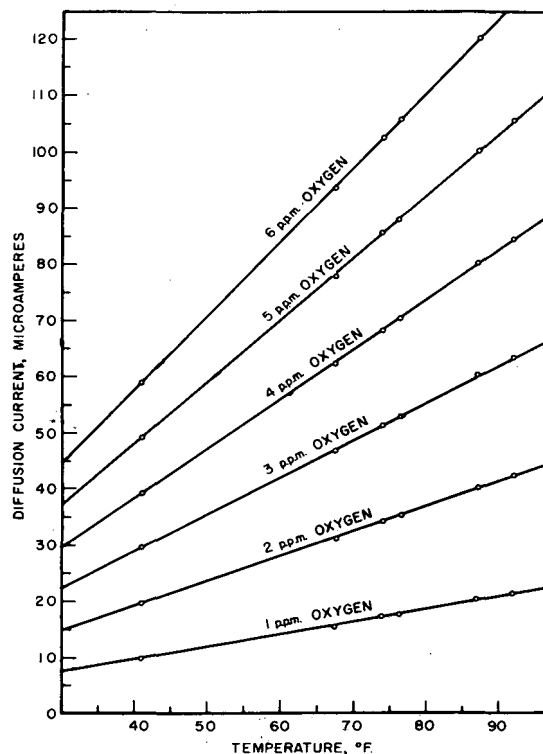Figure 8. Dissolved oxygen concentration vs. diffusion current



Figure 9. Diffusion current vs. temperature

designed for use with oil-field brines, all data presented herein were taken with the more rugged bright platinum electrode.

### CALIBRATION AND OPERATION

In making a dissolved-oxygen determination for calibration of the instrument, the brine was allowed to flow through the cell continuously. The sample for the Winkler determination was taken immediately after the amperometric determination was made, using standard field sampling technique (11). The rotating electrode was depolarized by applying a positive potential relative to the third, depolarizing electrode for a period of 10 to 20 seconds. Cleaning of the electrode was unnecessary until a visible amount of contamination had accumulated on its surface. The diffusion current was not read until approximately 15 seconds after the voltage was applied to the cell, to avoid the transient current indication that occurs immediately upon voltage application.

The total dissolved oxygen content was obtained by the following equation:

$$\phi = \frac{I - I'}{b[1 + a(T - T')]}$$

where

$\phi$ = dissolved oxygen concentration, p.p.m.
$I$ = total cell current, microamperes
$I'$ = residual current when $\phi$ = 0, microamperes
$b$ = electrode constant
$a$ = temperature coefficient
$T'$ = temperature at which temperature coefficient was determined, degrees Fahrenheit
$T$ = temperature of brine, degrees Fahrenheit

Construction of a simple nomograph of the type shown in Figure 7 as suggested by Marsh (7) simplifies use of the equation. The residual current, $I'$, is low enough to be neglected in practical considerations. Experiments showed that it ranged from 0.4 μa. at 40° F. to nearly 1 μa. at 100° F. No change in residual cur-

rent was noted with changes in dissolved-oxygen concentration, and it was assumed that the residual current varied only with changes in the electrode area, brine concentration, and temperature. The electrode constant, $b$, was computed from data obtained from one or two determinations on samples of brine with known oxygen concentrations. Rogers and others (8) were unable to find any close correlation between electrode area and diffusion current when using stationary platinum microelectrodes. The same difficulty was experienced by the writers when rotating microelectrodes were used. Possibly one reason for this trouble is the discrepancy between the calculated area and the actual effective area of the electrode because of minute cracks or pores in its surface.

In Figure 8 the diffusion current, measured in microamperes, is plotted against dissolved-oxygen concentration, in parts per million, at several temperatures. It is shown that diffusion current is a linear function of dissolved-oxygen content at constant temperature, within the range of temperature investigated.

Figure 9 shows the change in diffusion current with changes in temperature at a constant dissolved-oxygen concentration. This plot also is linear within the limits of experimental error. If it is assumed that the diffusion current is limited by the rate of ionic diffusion in the supporting electrolyte at the electrode, it is apparent that the rate of change of ionic diffusion with temperature is the major factor in the diffusion current–temperature coefficient. Kolthoff (5) shows that the ionic diffusion–temperature coefficient is equal to the equivalent conductance–temperature coefficient plus the reciprocal of the temperature. Figure 10 is a plot of equivalent conductance–temperature coefficient change with changes in temperature for different concentrations of sodium chloride. The plots were calculated from data given by Bremmer (1). Computation of the ionic diffusion–temperature coefficient from these data gives a value of change of 1.38% per degree Fahrenheit at 68° F. with a concentration of 50,000 p.p.m. of sodium chloride. The diffusion current–temperature coefficient was found experimentally to be 1.41% per degree Fahrenheit under these conditions, which
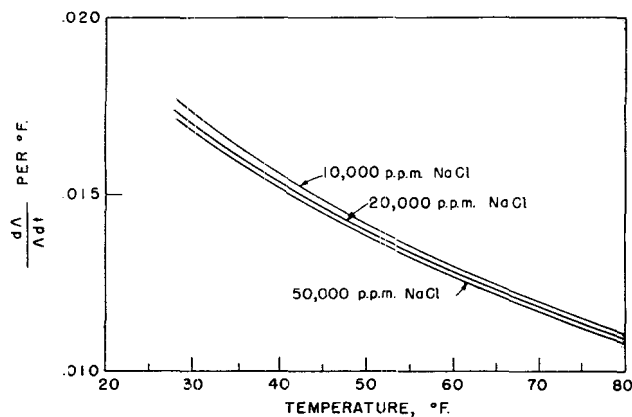
Figure 10. Equivalent conductance–temperature coefficient change with temperature, after Bremmer (1)

shows good agreement with the computed value. Thus, it may be seen that either close control or accurate measurement of the temperature is a necessity if reliable results are to be obtained.

Although not considered feasible in the construction of a single instrument, the use of a thermistor bridge, temperature-measuring device makes possible a self-compensating, direct-reading instrument. This could be done by adding a second variable resistance to the shaft of the thermistor bridge–balance element, thereby shunting the microammeter with a resistance of a value that would give a constant indication for a given oxygen concentration at all temperatures within the range of the instrument. A secondary shunt could be used to adjust the circuit for different electrode constants. This would eliminate the need for use of a nomograph in compiling results.

## CONCLUSIONS

The amperometric instrument for the determination of dissolved oxygen in brines, using a rotating platinum microelectrode, is capable of accurate, reproducible results when properly ap-

plied by the operator. Careful workmanship in the preparation of the electrode and diligent application is necessary if accuracy within about 5% is desired. When the meter is used for the first time to analyze brines that have not been tested before, comparison should be made with other methods, as the amperometric instrument may be sensitive to some ions present. The platinized electrode may prove useful with pure solutions in laboratory experiments where longtime stability is needed.

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# Determination of Decaborane

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Previous methods for the assay of decaborane have required the conversion of the borane to boric acid and determination of the latter compound. In addition to the possibility of losses involved in such an indirect procedure, the accurate analysis of micro quantities of boric acid has proved to be difficult. The methods described in this paper obviate these difficulties by permitting the direct determination of decaborane. A solution of decaborane in aqueous triethanolamine strongly absorbs ultraviolet light in the range 265 to 270 m$\mu$. Decaborane dissolved in xylene forms a soluble red adduct with quinoline which absorbs most strongly at 490 m$\mu$. Such solutions follow Beer's law, and from 1 to 25 $\gamma$ of boron per milliliter can be determined conveniently. The methods have been employed for analysis of air containing decaborane, by static and dynamic procedures, and for the assay of solid decaborane.

THE boron hydrides, or boranes, were laboratory curiosities for many decades, but have of late emerged as semiindustrial chemicals. No specific analytical procedures have ever been published for them, except a semiquantitative method of monitoring (1) which is based on principles and procedures which have been in use in these laboratories since January 1951.

Stock's classical book on the boron hydrides (5) discloses several methods for their analysis which depend on the hydrolytic conversion of the boranes to boric acid and hydrogen or the complete destruction of the molecules to boron and hydrogen. Schlesinger and Burg (3) also refer to hydrolysis, and Schlesinger and Schaeffer (4) disclose an alcoholysis (methyl) of pentaborane to methyl borate and hydrogen. Hurd (2) refers only to hydrolysis as a means of analyzing boron hydrides.

The methods based on hydrolysis or alcoholysis are burdened by the necessity of determining boric acid, which has always been one of the toughest problems of analytical chemistry. Where sizable quantities are involved, the well known titration method

is applicable and gives satisfactory results. How-
ever, trace quantities are not determined with ease,
nor with great accuracy, no matter whether the
turmeric, quinalizarine, carmine, or benzoin method
is used. The very voluminous literature on this
subject bears testimony to the difficulties involved.

The present paper gives two methods by which
microgram amounts of decaborane can be deter-
mined directly and quantitatively.

### ULTRAVIOLET METHOD

When boranes are dissolved in aqueous solutions
of alkali hydroxides, saltlike compounds are formed
which have been referred to (5) as hypoborates—e.g.,
sodium hypoborate (NaOBH$_3$). Such solutions ab-
sorb strongly in ultraviolet light, and this fact has
been made the basis for a direct determination of at
least one of the boron hydrides: decaborane. The
phenomenon is not restricted to solutions of deca-
borane in aqueous alkali but has also been ob-
served in other alkaline media, such as solutions in
aqueous ammonia, triethanolamine, diethanolamine,
monoethanolamine, morpholine, glycine, and in
nonalkaline media, such as chloroform, cyclohexane,
methanol, ethyl alcohol, and even water. However, the quanti-
tative work reported here is restricted to observations made on
solutions of decaborane in aqueous sodium hydroxide and tri-
ethanolamine. Work with other inorganic and organic alkaline
materials is in progress and will be reported at a later date.



Figure 1. Ultraviolet absorption of decaborane solutions and their
blanks at various wave lengths

### Table I. Hydrolysis of Decaborane Solutions with Respect to Time

| Solution A | | Solution B | |
|---|---|---|---|
| Min. after dilution to this concn. | % transm. at 270 m$\mu$ | Min. after dilution to this concn. | % transm. at 270 m$\mu$ |
| In Sodium Hydroxide | | | |
| 19 | 14 | 4 | 14 |
| 55 | 15 | 22 | 19 |
| 96 | 16.5 | 54 | 22.25 |
| 177 | 18.75 | 87 | 25.75 |
| 237 | 19.5 | 165 | 36.5 |
| 404 | 26.75 | 227 | 43.5 |
| 1354 | 66 | 321 | 60 |
| | | 392 | 67.75 |
| | | 1342 | 97 |
| In Triethanolamine | | | |
| 20 | 15.25 | 10 | 15.25 |
| 200 | 16.5 | 185 | 16 |
| 300 | 18 | 295 | 16.75 |
| 1380 | 27 | 1355 | 20.5 |
| 2925 | 38.25 | 2910 | 25.5 |

**Procedure.** For establishment of a standard curve, 0.0565
gram of decaborane was weighed out on a watch glass, washed
into a boron-free beaker with water, 15 ml. of a 1 to 1 (by vol-
ume) aqueous solution of triethanolamine were added, and the
mixture was allowed to stand for about 1.5 hours. The initially
yellowish solution was then diluted with water to 1000 ml. It
was used as a stock solution and contained 50 $\gamma$ of boron per
milliliter. Within 15 minutes the various dilutions were made
up, ranging from 2.5 to 25 $\gamma$ of boron per milliliter, and readings
of transmittance were obtained on these solutions within another
15 minutes (water as a blank).

For a comparison of the speed of hydrolysis of decaborane in
aqueous sodium hydroxide and triethanolamine, two samples of
0.0565 gram of decaborane were dissolved in 80 ml. of 0.6N
sodium hydroxide, or in 15 ml. of 1 to 1 triethanolamine. The
solution was diluted to 1000 ml. after 1 to 1.5 hours (Solution A,
containing 50 $\gamma$ of boron per milliliter); 25 ml. of Solution A was
diluted further to 50 ml. (Solution B, containing 25 $\gamma$ of boron
per milliliter) for reading. Solutions A and B were allowed to
stand for the number of minutes specified in Table I. Solution B
was then read directly and Solution A after dilution to 25 $\gamma$ of
boron per milliliter.
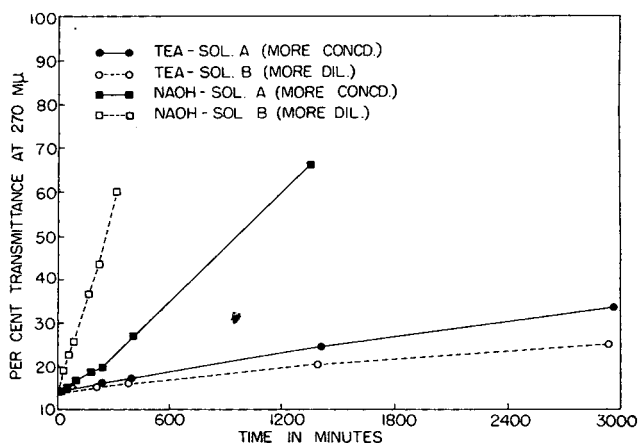


Figure 2. Hydrolysis of decaborane in aqueous sodium
hydroxide and aqueous triethanolamine

**Discussion and Results.** In general, absorption seems strong-
est at about 265 to 270 m$\mu$, as can be seen from Figure 1 (Beck-
man DU spectrophotometer used). The data for this figure are
strictly qualitative and comparisons of the various media cannot
be made because equivalent amounts of reagents were not used.

The decaborane used in this work was of reasonable purity,
having been purified (by the supplier) by sublimation. Its
melting point was 96.5° C., as compared with the figure of
99.7° C. given in the literature (3).

HYDROLYSIS. Solutions of decaborane in aqueous alkaline
media gradually hydrolyze with the formation of borates and hy-
drogen, the rate of hydrolysis depending on the kind of base em-
ployed and on the concentrations of decaborane and base. Solu-
tions of decaborane in sodium hydroxide deteriorate much more
rapidly than those in triethanolamine—more rapidly in dilute
than in more concentrated solutions—while decaborane solutions
in aqueous triethanolamine hydrolyze very slowly—more rapidly
in concentrated than in dilute solution. These results are ob-
served when strong and weak solutions are analyzed concurrently.
However, reproducibility in the rate of hydrolysis is not satis-
factory if solutions of the same concentrations are analyzed on
different days. This is probably due to temperature variations
which it was not possible to control because of lack of facilities.
Figure 2 shows typical pairs of curves of transmittance vs. time.

It was thought advisable not to plot all of the available data because the resulting closely spaced curves would have been nearly indistinguishable. However, Table I contains some of the transmittance data on hand, for 50- and 25-γ boron concentrations on which most of the work was done.

It is obvious from a study of Table I and Figure 2 that for quantitative work preference must be given to triethanolamine as the base in which to dissolve decaborane. Such solutions hydrolyze so slowly that plenty of time is available for any reasonable manipulative steps in an assay. If final readings are made on the spectrophotometer within 5 hours from the time of dissolving the decaborane, the results show an average deviation of only 1 to 2% at most decaborane levels.

Figure 3 shows a standardization curve for solutions containing 2.5 to 25 γ of boron (as decaborane) per milliliter. It is substantially a straight line, which indicates that the solutions follow Beer's law. Table II gives the instrument readings on which this curve is based.



**Figure 3.   Standardization curve for determination of decaborane in triethanolamine**

Beckman DU spectrophotometer

### Table II.   Standard Curve of Decaborane in Triethanolamine vs. Water Measured at 270 mμ

| Boron, γ per Ml. | % Transm. at 270 mμ | | | |
|---|---|---|---|---|
| 25 | 14.75 | 15 | 15 | 16 |
| 20 | 21.75 | 22 | 21.75 | 22.25 |
| 15 | 32.25 | 32.25 | 32 | 33.5 |
| 10 | 47 | 47 | 46.75 | 48 |
| 5 | 69.25 | 68.5 | 68 | 69 |
| 2.5 | 83.75 | 82.75 | 82.5 | 83.5 |

### Table III.   Purity of Decaborane after Prolonged Contact with Laboratory Air

| Time of Standing before Dilution, Hours | Boron Dilution, γ per Ml. (Nominal) | % Transmittance at 270 Mμ | Boron Found, γ per Ml. | Purity of Sample with Respect to Purity of Original Material, % |
|---|---|---|---|---|
| 1.25 | 15 | 33.5 | 14.5 | 96.7 |
| 1.25 | 15 | 33 | 14.7 | 98 |
| 2 | 10 | 46.5 | 9.9 | 99 |
| 2 | 10 | 47 | 9.8 | 98 |

A sample of decaborane which had been used for vapor analysis work, and through which undried laboratory compressed air and water-pumped cylinder nitrogen had been passed repeatedly, was analyzed by the present method. It had been surmised that frequent contact with moisture of the gases passed through the sample had converted the decaborane partially to boric acid. Four separate batches of 0.0565 gram each were dissolved in 1 to 1 aqueous triethanolamine as outlined, and the solutions were allowed to stand for various periods of time before dilution. Dilutions were made to represent 10 and 15 γ of boron per milliliter if the unknown had been 100% decaborane (nominal concentrations). The results are shown in Table III.

VAPOR ANALYSIS. The applicability of the new method to decaborane vapor analysis was tested in the following manner:

Several grams of decaborane were placed in a Turner absorption bulb (Corning No. 1960), the head of which was stuffed with glass wool to prevent carry-over of solid decaborane by the gas stream. Air or water-pumped nitrogen at a rate of 0.5 liter per minute was passed through this "generator" into an absorption train consisting of two test tubes with Vigreux-type indentations and each containing 30 ml. of 1 to 1 aqueous triethanolamine. Inlet and outlet of the Turner bulb carried ball and socket joints, respectively, and the "Vigreux bubblers" had standard taper heads with inlet and outlet tubes carrying ball and socket joints. Rubber connections must be avoided in all work with boranes because rubber absorbs boron hydrides avidly, leading to serious losses and embrittlement of the rubber. The apparatus is sketched in Figure 4.
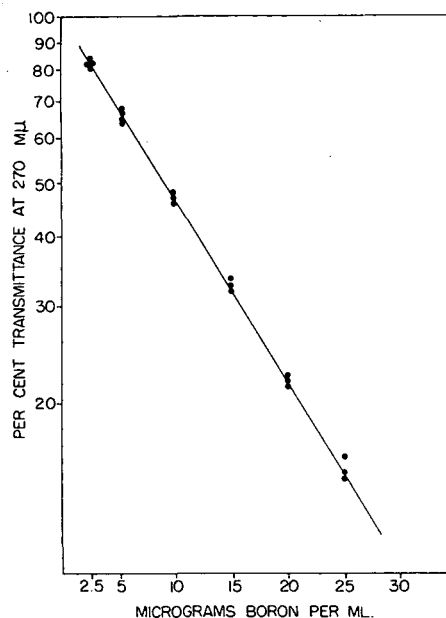
At the end of the test period, usually about 4 hours, the Turner bulb was weighed to ascertain the loss of decaborane during the test period, the contents of the Vigreux bubblers (the second one contained less than 0.3% of the total, on an average) were united and diluted appropriately, and the ultraviolet absorption of the final solutions was determined in a Beckman DU spectrophotometer.

Table IV gives the recoveries obtained. At first sight these results seem confusing and erratic. However, when the recoveries are plotted against the amounts of decaborane vaporized it becomes apparent that the errors, in general, become smaller as the quantity of decaborane vaporized in a run increases. Clearly, this points to weighing errors which are understandable in view of the great weight (about 100 grams) and large surface of the generator (Turner bulb).

This supposition does not explain the several very large deviations. It may be suggested that they were caused either by the volatilization of impurities (largest deviations generally observed after a generator was just filled with fresh material) or by the formation of a boric acid coating on fresh decaborane crystals (moisture and/or oxygen of the air) which would camouflage the actual decaborane losses from the generator. The more than fivefold increase in weight when decaborane is converted to boric acid (equivalent weights 122 to 618) makes this assumption entirely plausible, and the purity determinations on decaborane



**Figure 4.   Apparatus used in dynamic air analysis**

## Table IV. Dynamic Sampling of Decaborane Containing Gases by Triethanolamine-Ultraviolet Method

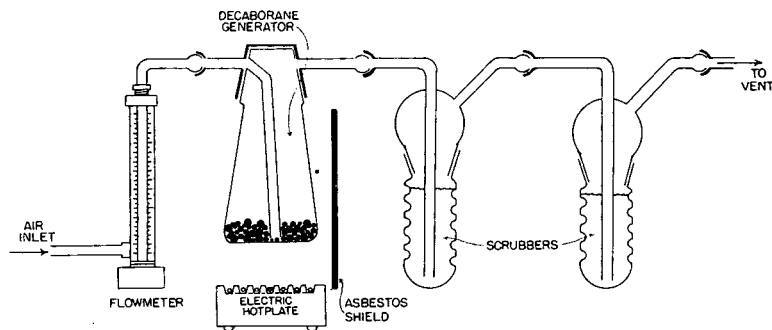| Gas Used | Decaborane, Mg. Vaporized | Found | Recovery, % |
|---|---|---|---|
| Air | 8.7 | 28.2 | 324[a] |
| | 37.1 | 39.3 | 106 |
| | 42.6 | 41.9 | 98 |
| | 33.1 | 30.5 | 92 |
| Nitrogen | 25.7 | 22.4 | 87 |
| | 56.4 | 55.8 | 99 |
| | 19.2 | 20.3 | 106 |
| | 48.3 | 51.8 | 107 |
| | 11.5 | 9.4 | 82 |
| | 10.9 | 8.9 | 82 |
| | 23.2 | 18.4 | 79 |
| | 27.4 | 28.2 | 103 |
| Air | 6.8 | 19.5 | 287[a] |
| | 1.8 | 19.4 | 1078[a] |
| | 43.3 | 44.3 | 102 |
| Nitrogen | 32.2 | 19.4 | 60[a] |
| Air | 29.2 | 30.5 | 104 |
| Nitrogen | 16.9 | 16.2 | 96 |
| Air | 24.3 | 26.5 | 108 |
| Nitrogen | 22.0 | 19.9 | 90 |
| Air | 32.7 | 32.8 | 100 |
| Nitrogen | 23.9 | 24.0 | 100 |
| Air | 33.4 | 32.7 | 98 |
| Nitrogen | 20.2 | 22.5 | 111 |
| Air | 27.3 | 29.7 | 109 |
| Nitrogen | 27.4 | 24.3 | 89 |
| | | Average | 97.6 |

[a] Not counted in the average.

## Table V. Dynamic Sampling of Decaborane-Air Mixtures by the Triethanolamine-Ultraviolet Method

| Decaborane, Mg. Vaporized | Found | Recovery, % |
|---|---|---|
| 94.8 | 97.7 | 103.1 |
| 102.3 | 94.0 | 92[a] |
| 110.9 | 112.9 | 101.8 |
| 127.8 | 132.0 | 103.3 |
| Average | | 102.7 |

[a] Not counted in average.

from repeatedly used generators given in Table III lend further credence to this supposition.

In view of these considerations, another series of tests was made in which enough decaborane (about 100 mg.) was vaporized to minimize weighing errors. In conducting a test the generator was weighed initially, air passed through it, the decaborane vapor caught in aqueous triethanolamine, and the solution analyzed twice a day (each time with fresh triethanolamine solution). The individual recoveries were added up, and the generator was weighed again at the end of each complete run. To speed up vaporization of decaborane, and thus save time, a hot plate was placed under the generator at a distance of about 5 inches to bathe it in a current of warm air (after the first run). The temperature of the decaborane (not measured) was raised to perhaps 30° to 35° C. in this manner. Results obtained (see Table V) show excellent agreement and prove that this method is dependable for air analysis of decaborane.

In Table V, the low recovery of 92% is readily explained. It was obtained in the first run in which the hot plate was used. As the latter was not shielded from the triethanolamine scrubbers, their contents warmed up considerably with resultant faster hydrolysis and greater transparency of the solution in the ultraviolet. This would simulate a lower recovery. An asbestos shield was used in all succeeding runs and no more trouble was encountered.

Recoveries above 100% in the other three runs (Table V) are also readily explained. The decaborane used was not of 100% purity, as evidenced by its melting point. The standardization curve was obtained with this less than 100% pure material. During the test, however, only pure decaborane vapor is released and impurities like boric acid probably are not vaporized. Thus, recoveries can be above 100%, when the solutions are read against a standardization curve obtained with material of less than 100% purity.

AIR SAMPLING. While the present method was developed primarily for the analysis of solid decaborane and mixtures containing it, and for the dynamic analysis of decaborane-contaminated air with a special view to plant monitoring, it is seemingly also applicable to static air sampling. Preliminary tests were made in which air samples (loaded with decaborane vapor at room temperature) were taken with a 250-ml. gas pipet into which 5 ml. of aqueous triethanolamine solution were then introduced by means of a Yale syringe. Decaborane concentrations of about 50 p.p.m. were thus found, a fact which is in line with findings during dynamic sampling. However, limitations of the static sampling method are yet to be worked out and are to be published as data become available.

Both diborane and pentaborane dissolve in aqueous triethanolamine with the formation of compounds which absorb light in the ultraviolet region. However, in the case of diborane, decomposition is so fast that for practical purposes the residual opacity will be of no consequence, and in the case of pentaborane retention by the aqueous amine (in a dynamic air analysis) is so poor and decomposition of the solution so fast that interference should be negligible.

### COLORIMETRIC METHOD

While the above ultraviolet method is satisfactory, it has two inherent disadvantages. It requires instrumentation which is not available in every industrial plant and laboratory, and it entails the analysis of solutions which undergo constant changes due to hydrolysis. The development of another method, free of these drawbacks, seemed therefore most desirable.
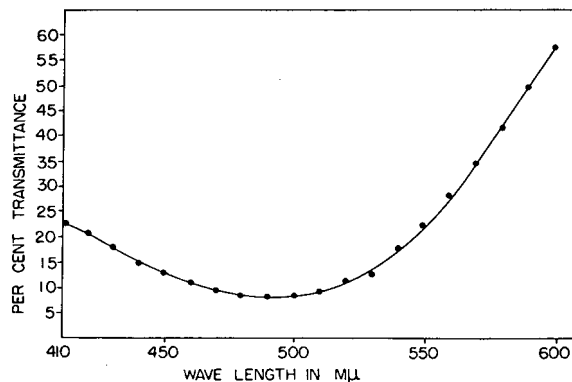


Figure 5. Absorption of solution of decaborane in xylene containing 2 volume % of quinoline

Coleman Junior spectrophotometer

It was found that decaborane combines with quinoline to form a red compound which lends itself to colorimetric analysis, showing maximum absorption at 490 m$\mu$ (see Figure 5). The reaction is best carried out in an aromatic solvent, such as xylene or benzene, because the new compound is soluble therein, but the reaction also takes place in a number of other solvents. It does not occur in alcoholic media, such as methanol or ethyl alcohol, nor in water. Decaborane dissolved in aqueous sodium hydroxide or triethanolamine likewise gives no color with quinoline. In ethyl ether or ethyl acetate no color is obtained immediately, but an orange and light yellow color, respectively, are developed on standing overnight. Dissolved in ligroin, cyclohexane, or carbon tetrachloride, decaborane will react with quinoline, but the compound formed is almost insoluble in these solvents and is thus obtained as a brick-red powder. In this manner the compound can be isolated and purified for analysis, and results thus obtained are given in Table VI. The figures indicate that decaborane and quinoline combine in a ratio of 1 to 1.

**Procedure.** A standard curve is obtained in the following way. Five milliliters of C.P. quinoline are added to approximately 100 ml. of C.P. xylene contained in a 250-ml. volumetric flask. A sample of 0.0565 gram of decaborane (0.0500 gram of boron) is dissolved in xylene in a beaker and is washed quantitatively into the volumetric flask. A very deep red color develops almost immediately. The flask is diluted to volume with xylene (Standard A = 200 γ of boron per milliliter), and the following dilutions are made with xylene:

Standard B. 5 ml. of Standard A diluted to 50 ml. (20 γ of boron per milliliter)

Standard C. 5 ml. of Standard A diluted to 100 ml. (10 γ of boron per milliliter)

Standard D. 5 ml. of Standard A diluted to 250 ml. (4 γ of boron per milliliter)

Standard E. 25 ml. of Standard D diluted to 100 ml. (1 γ of boron per milliliter)

The solutions should stand for about 1.5 hours before readings are taken, or repeated measurements should be made until greatest color density is obtained. To eliminate the influence of the color which quinoline always has (unless freshly distilled), blank solutions are prepared in exactly the same manner as Standards A through E, except that no decaborane is added. Old and darkened quinoline should be redistilled before use.
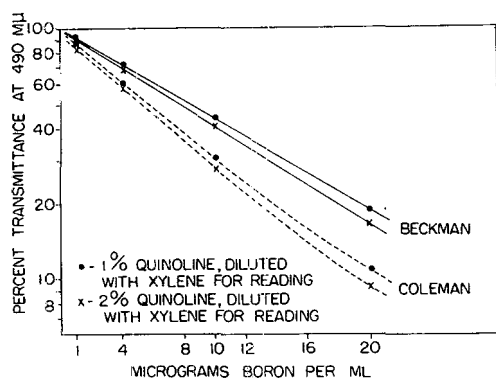


**Figure 6. Standardization curves for determination of decaborane by the quinoline method**

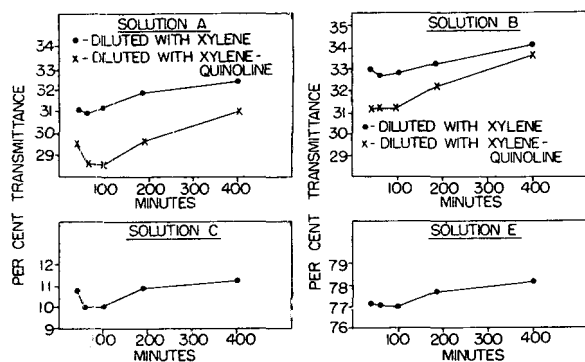Beckman DU spectrophotometer and Coleman Junior spectrophotometer



**Figure 7. Time necessary for maximum development of quinoline-decaborane color**

**Discussion and Results.** BEER'S LAW. Solutions of the decaborane-quinoline compound follow Beer's law strictly when measured in the Beckman DU spectrophotometer, though readings deviate slightly from a straight line at higher concentrations in a less linear instrument such as the Coleman Junior spectrophotometer (see Figure 6). Full linearity might not be obtained if the solutions are read too quickly, about 1.5 hours being necessary for maximum color development. Table VII and Figure 7 point this out.

REAGENT CONCENTRATION. Figure 6 shows standard curves obtained with the Beckman and Coleman instruments, respec-

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**Table VI. Analysis of Decaborane-Quinoline Compound**

| Element | Found, % | Theory for 1 to 1 Mole Addition, % |
|---|---|---|
| N | 5.45, 5.20 | 5.58 |
| C | 44.06 | 43.03 |
| H | 6.84 | 7.23 |

**Table VII. Time Necessary for Maximum Development of Quinoline-Decaborane Color**

| Minutes Elapsed from Time Solution Made to Time Read | Transmittance, % | | | | Solution C (not further diluted) [c] | Solution E (not further diluted) [d] |
|---|---|---|---|---|---|---|
| | Solution A [a] | | Solution B [b] | | | |
| | Diluted with xylene | Diluted with xylene-quinoline | Diluted with xylene | Diluted with xylene-quinoline | | |
| 40 | 31.1 | 29.5 | 32.9 | 31.2 | 10.7 | 77.1 |
| 60 | 31.0 | 28.7 | 32.7 | 31.2 | 10.0 | 77.1 |
| 95 | 31.2 | 28.6 | 32.8 | 31.2 | 10.0 | 77.0 |
| 185 | 31.9 | 29.7 | 33.2 | 32.2 | 10.8 | 77.7 |
| 400 | 32.4 | 31.0 | 34.1 | 33.6 | 11.2 | 78.2 |

[a] Solution contained 200 γ of boron per milliliter in xylene containing 2% quinoline; since it was too concentrated to be read directly, it was diluted to 10 γ of boron per milliliter for reading, in one case with xylene and in another case with 2% xylene-quinoline reagent.

[b] Solution contained 100 γ of boron per milliliter in xylene containing 2% quinoline; since it was too concentrated to be read directly, it was diluted to 10 γ of boron per milliliter for reading, in one case with xylene and in another case with 2% xylene-quinoline reagent.

[c] Solution contained 20 γ of boron per milliliter in xylene containing 2% quinoline.

[d] Solution contained 2 γ of boron per milliliter in xylene containing 2% quinoline.

**Table VIII. Standardization Curves for Decaborane-Quinoline Analysis**

(Comparison of Beckman and Coleman Junior spectrophotometer) [a]

| Boron, γ/Ml. | Transmittance, % | | | | | |
|---|---|---|---|---|---|---|
| | Coleman, after 2.25 Hours | | Beckman, after 2.5 Hours | | Coleman, after 4.5 Hours | |
| | 1% quinoline | 2% quinoline | 1% quinoline | 2% quinoline | 1% quinoline | 2% quinoline |
| 20 | 10.8 | 9.2 | 18.5 | 16.3 | 10.9 | 9.9 |
| 10 | 30.2 | 27.3 | 43.8 | 40.8 | 30.5 | 27.7 |
| 4 | 61.1 | 58.5 | 71.7 | 69.6 | 60.8 | 58.5 |
| 1 | 88.0 | 87.2 | 92.8 | 91.3 | 87.7 | 86.8 |

[a] Stock solutions made up with 1 and 2% quinoline in xylene, respectively, and dilutions made with xylene.

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tively, for 1 and 2% quinoline reagent, and Table VIII furnishes the underlying data.

The use of xylene for dilution of samples to readable levels might be objected to because it reduces the quinoline concentration. To ascertain the importance of this factor, one series of tests was made in which the quinoline concentration was kept at 2% at all times by diluting the stock solution with a 2% solution of quinoline in xylene, and another series was run in which xylene was used for diluting the stock solutions. Figure 8 and Table IX show the results obtained.

The concentration of quinoline in the reagent is of some importance, unless it is kept constant at all times and at about 2% quinoline or above. Table X indicates how the depth of color changes with varying quinoline content of the reagent. It also shows that the 2% concentration chosen arbitrarily for this investigation fortuitously came close to optimum conditions.

AIR ANALYSIS. Application of the new method to air analysis has given splendid results. The experimental setup was the same as that described for the ultraviolet method (Figure 4), except that the absorption train was charged with a 2% solution of quinoline in xylene. Absorption of decaborane from the air current (0.5 liter per minute sampling rate) was so efficient and complete that only one absorption tube was used after the initial experiments. Incidentally, it was found in this work that xylene itself is a most efficient scrubbing agent to absorb decaborane from an air current, though, of course, it does not produce a color. Table XI gives results obtained. An explanation of the fact that recoveries are generally above 100% has been given when discussing the ultraviolet method of decaborane analysis.

**Table IX.  Comparison of Xylene and 2% Quinoline in Xylene as Diluents**

| Dilution Material | Boron, γ/Ml. Sample | % Transmittance after 125 Minutes |
|---|---|---|
| Xylene | 20 | 10.2 |
| | 10 | 28.7 |
| | 4 | 58.9 |
| | 1 | 89.0 |
| 2% quinoline in xylene | 20 | 9.7 |
| | 10 | 30.3 |
| | 4 | 61.0 |
| | 1 | 87.0 |

**Table X.  Optimum Concentration of Reagent for Quinoline-Decaborane Tests[a]**

| Quinoline, % | % Transmittance after 115 Minutes |
|---|---|
| 20 | 33.6 |
| 10 | 30.3 |
| 5 | 30.1 |
| 2 | 30.5 |
| 1 | 32.5 |
| 0.5 | 38.9 |

[a] Final concentration of boron = 10 γ per ml.

**Table XI.  Analysis of Air-Decaborane Mixtures by Quinoline Method**

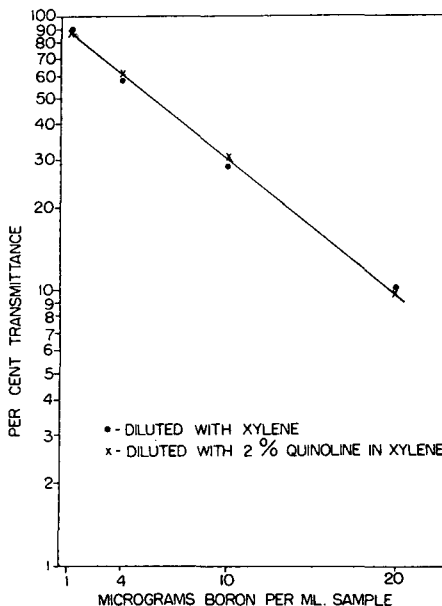| Decaborane, Mg. | | Recovery, % |
|---|---|---|
| Vaporized | Found | |
| 148.3 | 149.3 | 100.7 |
| 117.3 | 115.9 | 98.8 |
| 84.8 | 89.9 | 106.0 |
| 110.3 | 114.9 | 104.0 |
| Average | | 102.4 |

GAS SAMPLING.  Besides being useful for dynamic air analysis, the quinoline method is seemingly also applicable to static gas sampling.  In one test, nitrogen loaded with decaborane vapor at room temperature was passed through a 250-ml. gas pipet for 5 to 10 minutes.  Then 10 ml. of 2% quinoline reagent was introduced by means of a Yale syringe, and the pipet was shaken vigorously.  Nothing happened at first, but gradually a pink color developed.  After about 15 minutes, the liquid was drained from the gas pipet into a Coleman cuvette and readings were taken at irregular intervals.  Transmittance decreased from an initial 63.5% to a steady state of 44.9% which corresponds to 6.1 γ per ml.  Calculations made of the decaborane concentration in the air stream (from the weight loss of the generator and the average volume of the irregular nitrogen stream) indicated that the liquid in the gas pipet should have contained 4 to 6 γ per ml., and two independent gas samples analyzed by the ultraviolet method gave values of 6.0 and 5.5 γ per ml.  The conclusion therefore seems justified that the quinoline method of decaborane analysis is applicable to static gas analysis.

COLOR STABILITY AND FORMATION.  The decaborane-quinoline color is stable for a long time, only about 10% loss in color being experienced after 3 days.  The reason for this great stability must be sought in the nonpolar character of the solvent xylene which precludes hydrolysis of the borane complex.  Concentrations of about 1 to 20 γ of boron (as decaborane) per milliliter of solution can be determined conveniently.

The red color obtained with quinoline and decaborane is unique.  It is most remarkable that the introduction of some substituents into the quinoline ring destroys its ability to produce strong color with decaborane.  The hydroxyl group (in positions 2 or 8) will produce this change, as will the methyl group (quinaldine).  Isoquinoline produces an orange color.  Many other nitrogenous compounds have been tried, such as pyridine, piperidine, pyrrole, piperazine, benzidine, diethyl-1-naphthylamine, 2-mercaptobenzothiazole, benzimidazole, benzotriazole, and phenylhydrazine.  There were indications that most, if not all, of the materials reacted with decaborane, but the colors produced ranged from colorless through yellow to orange, and no advantage could

be seen over the intense color produced by the cheap and readily available quinoline.  However, a number of very interesting observations were made during this "range-finding" investigation which will be reported at a later date.  Naphthoquinoline is the only other material found so far which produces a red color as intense as quinoline does and it may, indeed, be a stronger color.



**Figure 8.  Influence of diluent on quinoline-decaborane color**

The quinoline-xylene reagent absorbs diborane quantitatively and seems to react therewith.  However, no significant color is developed, and the solid diborane-quinoline addition product obtained on evaporation of the xylene is of a very light tan color.  Very interestingly, this product turns a dark brick red on prolonged storage (several months).  Pentaborane is also absorbed readily by the quinoline reagent, but only a light orange-yellow color is produced which disappears completely after standing for about 1 hour.  It appears, therefore, that the quinoline reagent is specific for decaborane (among the common boranes).  This view is supported further by the finding that an assay of a very crude mixture of solid boron hydrides by the quinoline method showed substantially the same decaborane content as had been found repeatedly by sublimation on a fairly large scale.

**GENERAL CONCLUSIONS**

Both methods described above are dependable tools for the microdetermination of decaborane.  In general, the colorimetric method will be found preferable, especially for dynamic air sampling, because of the great stability of the quinoline-decaborane color and the greater simplicity of the required instrumentation.

On the basis of information now available, it is believed that neither diborane nor pentaborane would interfere with the determination of decaborane by either method, provided that the procedures are followed properly.

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# Determination of Microgram Quantities of Fluoride and Cyanide by Measurement of Current from Spontaneous Electrolysis

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Measurement of the current from an electrochemical cell which operates spontaneously may be used as a measure of one of the reactants in the cell. Cyanide can be determined in the cell Ag , NaOH $(0.1M)$ | Pt and fluoride in the cell Al | $CH_3COOH$ $(0.2M)$ | Pt. The simplicity of the methods, combined with good sensitivity in the microgram range, suggests their use in portable detection devices for hydrogen cyanide or hydrogen fluoride vapors in industrial atmospheres.

INTERNAL or spontaneous electrolysis, in which the cell reaction proceeds without any external applied potential, has been used for a long time as a technique in electrogravimetry. Such electrolyses represent a type of limited potential electrolysis, the choice of the electrodes allowing a certain degree of control over the available voltage. In ordinary controlled potential electrolysis when 100% current efficiencies are obtained, a definite relation is to be expected between the instantaneous current and the bulk concentration of the limiting reactant $(1)$. Calculations based on instantaneous current readings have been suggested as a substitute for the usual methods of current summation in controlled potential coulometric analysis $(2)$. The present work demonstrates that instantaneous current readings in spontaneous electrolysis may, under certain conditions, be an equally suitable measure of the concentration of the limiting reactant in solution. In practice, the accuracy of results based on this relationship may be insufficient for widespread application to the determination of major constituents, but entirely adequate for trace amounts where a greater relative error is usually permissible. Good results have been obtained in the determination of cyanide by means of a silver anode and fluoride with an aluminum anode. A platinum wire may be employed conveniently as cathode.
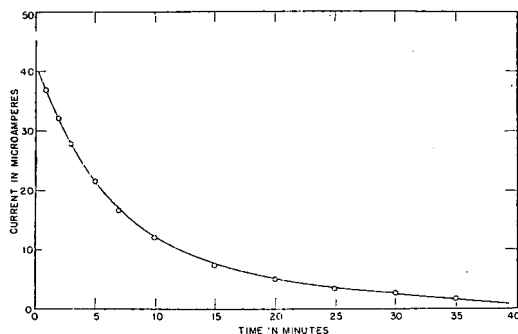


Figure 1. Current-time relationship in determination of cyanide

The use of a silver anode was suggested by analogy with the polarographic determination of cyanide by its effect on the mercury dissolution wave. The use of a silver electrode in the potentiometric titration of cyanide is also a well recognized procedure. In 1951, Roth $(3)$ presented a paper concerning the detection of hydrogen cyanide by measurement of the potential of a silver electrode. The existence of strong fluoride complexes of aluminum suggested the use of an aluminum anode for the detection of fluoride.

## EXPERIMENTAL

**Estimation of Cyanide.** Suitable electrodes consist of a spiral wound tightly from 18 inches of 18-gage silver wire, as anode and a similar spiral, from 10 inches of 0.03-inch diameter platinum wire, as cathode. The two spirals may be wound conveniently on the arms of a U-shaped, three-way, connecting tube (Kimble No. 45025) which provides the necessary permanent positioning of the electrodes. Sodium hydroxide $(0.1M)$ serves as the electrolytic solution. Stirring may be accomplished conveniently with a magnetic stirrer. Connection between the electrodes is made through a suitable precision microammeter (Weston Model 430, 0 to 200 $\mu a.$, 571 ohms internal resistance). When the electrodes are lowered into the hydroxide solution, a blank current of several microamperes flows momentarily but decreases to nearly zero in about 1 minute. If a few micrograms of cyanide in $0.1M$ sodium hydroxide solution are now added, a nearly exponential current-time curve similar to that in Figure 1 results.

The cell is calibrated by measurements on several standard solutions of cyanide, in the concentration range of interest, prepared in $0.1M$ sodium hydroxide. Five milliliters of $0.1M$ hydroxide are added to the cell and when the blank current has de-
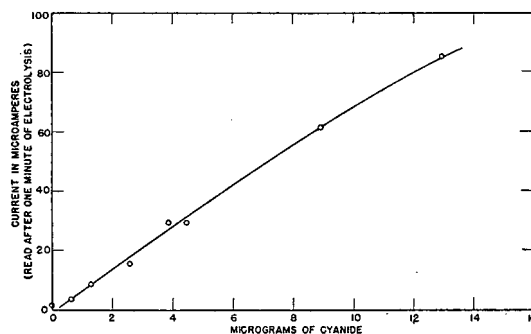


Figure 2. Typical calibration curve for determination of cyanide

creased to nearly zero, 5 ml. of the standard cyanide solution are added. After 1 minute the current is recorded. Then this cell solution is discarded and a similar procedure followed with another concentration of cyanide. A curve similar to that in Figure 2 results from these data.

The current is a function of the size of both electrodes (especially the anode), the cell dimensions, external resistance (microammeter), rate of stirring, and similar variables. The calibration curve often remains valid for days of use if none of these factors changes. However, changes sometimes occur for no apparent reason (probably the surface condition of the electrodes has been unknowingly altered) and therefore frequent checks are advisable. This is no serious handicap to the usefulness of the method, inasmuch as the process of recalibration is simple and rapid.

On continued use the sensitivity of the cell may show a slow, steady decrease. In this case cleaning of the platinum cathode with hot nitric acid often has been found to restore the cell to nearly its original sensitivity. Cleaning of the silver seems unnecessary and even inadvisable, because silver anodes cleaned in acid exhibit erratic behavior for some time. Silver wire from several sources was used as received and little variation was noted. Ordinary silver wire, such as is supplied for medical purposes, seems to serve well. If the electrodes have stood for some while without use, making several measurements with a standard

cyanide solution until reproducible values are obtained has been found to be a good way to clean and condition the electrodes.

This device is admirably suited to the detection of hydrogen cyanide in air, since $0.1M$ sodium hydroxide serves well as a collection medium and then may be transferred directly to the cell. The cell may be used to monitor atmospheres for hydrogen cyanide by leading the air stream through the U-shaped connecting tube on which the electrodes are wound. In this case the magnetic stirrer is unnecessary as sufficient stirring is provided by the air stream. Any increase in current above the base line (nearly zero) indicates the presence of cyanide and the rate of increase is a measure of the amount of cyanide present in the air.

In the presence of air the following chemical reaction may take place in the cell and result in consumption of cyanide and consequently smaller currents from the electrochemical reaction:

$$8CN^- + O_2 + 4Ag + 2H_2O \longrightarrow 4Ag(CN)_2^- + 4OH^- \quad (1)$$
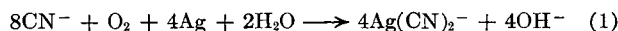
This reaction apparently occurs to an appreciable extent in solutions $2 \times 10^{-4}M$ in cyanide and $0.01M$ in hydroxide. However, in 0.1 or $1M$ hydroxide and $2 \times 10^{-5}M$ or lower cyanide concentration, little or no loss of cyanide results by this reaction.

**Table I. Effect of Various Substances on Estimation of Cyanide by Silver Anode**

| | | Current, Read after 30 Sec., µa. | |
| Substance Added | Equivalent to 1000 γ of | Blank current | Response current, to 2.6 γ of CN⁻ |
|---|---|---|---|
| None | .... | 0.7 | 17.7 |
| NaNO₃ | NO₃⁻ | 0.5 | 17.3 |
| NaNO₂ | NO₂⁻ | 0.6 | 17.7 |
| NaF | F⁻ | 0.7 | 17.0 |
| HCl | Cl⁻ | 0.6 | 17.0 |
| Na₂SO₃ | SO₃⁻⁻ | 0.7 | 16.6 |
| H₂SO₄ | SO₄⁻⁻ | 0.7 | 16.9 |
| Na₃PO₄ | PO₄⁻⁻⁻ | 0.7 | 17.0 |
| NH₄OH | NH₃ | 0.0 | 17.0 |
| NaOCl | OCl⁻ | −12.0 | 3.6 |
| Na₂S | S⁻⁻ | Off scale positive | .. |

The effect of possible atmospheric contaminants on the behavior of the cell was investigated. Inasmuch as the electrolytic solution is concentrated sodium hydroxide, the effect of acidic gases could be determined conveniently by adding their sodium salts. Approximately 1000 γ of the various substances (contained in a few tenths of a milliliter of solution) were added to a cell containing 10 ml. of $1M$ sodium hydroxide solution. When no added substance was present, the cell showed a blank current (read after 30 seconds) of 0.7 µa. and a response current of 17.7 µa. to 2.6 γ of cyanide. Table I shows the values obtained in the presence of various contaminants. Of those tried, only hypochlorite (which would be obtained from chlorine) and hydrogen sulfide appear to be serious interferences at the 1000-γ level. Five micrograms of hypochlorite gave no interference. Five micrograms of sulfide produced a blank current of 3 µa. In both cases, subsequent addition of 2.6 γ of cyanide gave the usual additional 17 to 18 µa. of current.

**Determination of Fluoride.** A cell similar to that described for cyanide may be used, with the exception that the anode should be aluminum wire of considerably smaller surface area than was the silver anode. A straight piece of 0.125-inch diameter, 99.99% aluminum wire (Aluminum Co. of America) was found to be suitable. Ordinary aluminum (99.7%) can be used, but somewhat higher and more irregular blank currents are obtained. The wire should be coated where it enters the solution with a nonconducting film (Apiezon hard wax W is suitable) to avoid erratic current readings due to a variable surface exposed to the solution. An exposed length of about 0.5 inch was suitable. Acetic acid $(0.2M)$ serves satisfactorily as the electrolytic solution.

When the electrode is immersed in the solution, a relatively large current flows momentarily and decreases to a constant value in 3 or 4 minutes. This blank current varies with the size and condition of the electrode, but usually is 5 to 10 µa. If fluoride is now added to the cell, the current rapidly increases and then decreases in a regular fashion, as shown in Figure 3. The magnitude of this current is proportional to the amount of fluoride present, as is indicated by Figure 4, where current values, read after 2 minutes, are plotted against micrograms of fluoride present.

A nearly saturated solution of benzoic acid $(0.017M)$ may be used instead of the $0.2M$ acetic acid. Benzoic acid gives a much smaller blank current and definitely is preferred when small amounts of fluoride (less than 5 γ) are being determined.

As with the silver anode, the current is a function of rate of stirring, electrode size, and, to a certain extent, history of the

electrode (probably to the extent that its history determines the condition of the surface). Therefore, determination of a calibration curve is necessary under the exact conditions of the analysis. The simplicity of the procedure makes this task easy. Cleaning and conditioning the aluminum for 2 minutes in $0.01M$ hydrofluoric acid before beginning a series of determinations are advisable.

Nitrate, sulfate, and cyanide produced no interference in 1000-γ amounts. Phosphate and sulfide cause some variation, but probably could be compensated by calibrating the cell with approximately the expected amounts of these anions present. Chloride (1000 γ) causes large currents and is a serious interference at this concentration.
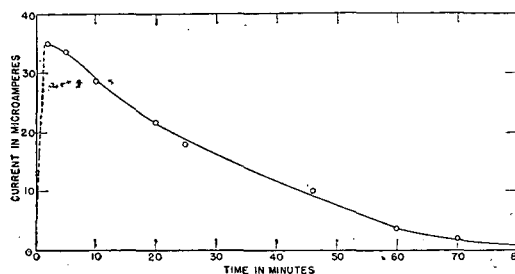


**Figure 3. Current-time relationship in determination of fluoride**
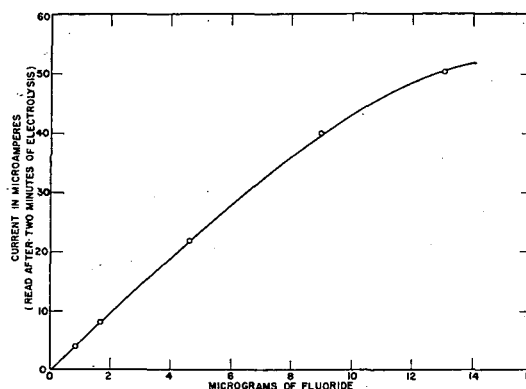


**Figure 4. Typical calibration curve for determination of fluoride**

Inasmuch as the electrode shows good response to 1 p.p.m. of fluoride, the method may be useful for determining fluoride in fluoridated public water supplies in cases where large amounts of chloride are not present.

### ACKNOWLEDGMENT

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# Microchemical Detection of Characteristic Functional Groups in Steroids

## Side Chains

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Procedures for the qualitative differential determination of steroid functional groups are useful in the fields of chromatography and organic chemical analysis. Micromethods have been devised to elucidate the side chain structures of adrenal cortical and sex hormones, using 60 $\gamma$ or less of each compound. Periodic acid, lead tetraacetate, acetic anhydride, chromium trioxide, lithium aluminum hydride, and aluminum isopropoxide, together with triphenyltetrazolium chloride and *m*-dinitrobenzene, have been applied for the identification of nine side chains. These tests will be found an aid to the identification of steroid compounds found in micro quantities.

N UMEROUS color tests have been devised in the field of paper chromatography (*1–6*, *10–14*, *16–19*, *22–25*) for the detection of steroid compounds. Most of these are not specific and may give positive reactions with any steroid which contains one or more hydroxyl or ketone functional groups. Two of the above tests which are applicable to steroid side chains, however, are fairly selective. The dye, triphenyltetrazolium chloride (*5*), reacts in alkaline solution with α-ketol and dihydroxyacetone reducing side chains to form a red complex; also, Zimmermann's reagent as modified by Kochakian (*13*) and Axelrod (*3*) gives a violet color with 17-ketones and a blue color with 3-ketones. (By convention, α-ketol and dihydroxyacetone side chains are designated, respectively, as 20-keto, 21-hydroxy, and 20-keto, 17,21-dihydroxy groupings.)

This paper describes methods of sequentially applying known chemical reactions to micro quantities (15 $\gamma$) of steroids on filter paper and thereby identifying from the derivatives the known side chains of adrenocortical and sex hormones. These reactions will be found helpful in the field of paper chromatography for the identification of micro quantities of unknown steroids and in steroid chemistry for the rapid identification of side chains in synthetic or analytical procedures.

### MATERIALS AND REAGENTS

Hot Plate (Lindberg), with stepless control. (Unless otherwise stated, the surface temperature is about 80° C.)

Glass Plates, approximately 3 × 5 inches.

Glass Rods, approximately 4 inches long and $^1/_8$ inch in diameter.

Porcelain Evaporating Dishes, 6.0 to 7.5 cm. in diameter.

Filter Paper, Whatman No. 1.

Reagents. A. PERIODIC ACID, a 3% solution of periodic acid (G. Frederick Smith Chemical Co.) in methanol-water (1 to 1 by volume). This reagent is stable for at least 3 months in a brown bottle.

B. CHROMIC ACID, a 0.4% solution of c.p. chromium trioxide in 90% acetic acid. The solution is usable until the reddish brown color begins to show greenish overcasts.

C. LEAD TETRAACETATE, a saturated solution of lead tetraacetate (*8*) in 19 volumes of glacial acetic acid and 1 volume of acetic anhydride. The reagent is stable for months in a tightly stoppered brown bottle.

D. CHROMIUM TRIOXIDE–ACETIC ANHYDRIDE, a 0.2% solution of c.p. chromium trioxide in acetic anhydride. This reagent is usable only for 36 hours.

E. ALUMINUM ISOPROPOXIDE, a 1% solution of aluminum isopropoxide in toluene-cyclohexanone (1 to 1 by volume). This

modified Oppenauer reagent is not stable because of the decomposition of the cyclohexanone and must be prepared before use.

F. LITHIUM ALUMINUM HYDRIDE. Finely divided lithium aluminum hydride is suspended in absolute ether (approximately 1 to 2% "solution"). The suspension is prepared freshly before use.

G. TRIPHENYLTETRAZOLIUM CHLORIDE (TPTZ) (*5*), a 0.2% aqueous solution of triphenyltetrazolium chloride mixed just before use with an equal part of 3.5N aqueous sodium hydroxide.

H. DINITROBENZENE (*3*), 2 parts of a 1% methanolic solution of *m*-dinitrobenzene mixed with 1 part of aqueous 15% potassium hydroxide just before use.

### METHODS

Whatman No. 1 filter paper is cut into 3 × 1 inch strips and 15 $\gamma$ of the steroid is applied at one end of the paper on the smooth side, by means of a micropipet within a circle of approximately 0.7 cm. A stream of nitrogen is used intermittently to evaporate the solvent.

The various organic chemical reactions are carried out by immersion of the filter paper, bearing the steroid, into one of the reagents contained in a porcelain evaporating dish. The procedures used for the reactions are described below with alphabetical correspondence to the previously listed reagents.

A. The test strip is dipped into the periodic acid reagent and placed on a glass plate. The glass is then heated on the hot plate until the paper is dry. In this and all subsequent tests, the strip must have complete contact with the glass plate. An aid in keeping the filter paper flat is to apply glass rods at both ends of the paper (away from the spot) during the drying period.



STEROID SIDE CHAINS
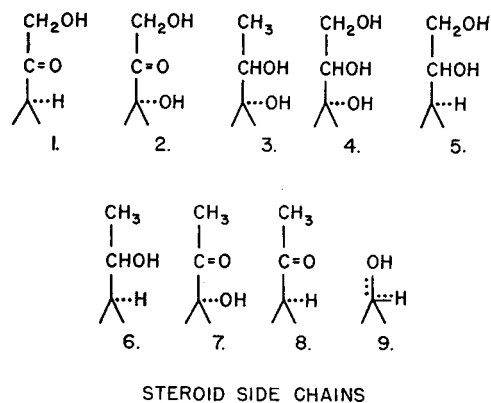
**Figure 1. Side chains of adrenocortical and sex steroids at carbon-17 detected by appropriately applied tests**

Only in side chain 9 are isomeric forms noted

B. A portion of the chromic acid solution is boiled, at which time the test strip is momentarily immersed in the boiling liquid and then dried on a glass plate preheated and still on the hot plate.

C. After a test strip has been dipped into the lead tetraacetate reagent, it is dried on the hot plate on a preheated glass plate until the paper turns light brown. The browning is due to the reduction of the excess lead tetraacetate by the moisture in the air. Usually the immediate area containing the unknown spot remains free of coloration.

```
                          1-9
                           |
                       Strip One
                           |
                           A
                           |
                           H
                     ┌─────┴─────┐
              Violet(3,4)      No color(1,2,5-9)
                  |                 |
              Strip Two         Strip Two
                  |                 |
                  D                 G
                  |           ┌─────┴─────┐
                  G      Red(1,2)       No color(5-9)
            ┌─────┴─────┐  |               |
         Red(4)   No color(3) Strip Three  Strip Three
                           |                 |
                           F                 F
                           |                 |
                           A                 A
                           |                 |
                           H                 H
                     ┌─────┴─────┐     ┌─────┴─────┐
                 Violet(2)  No color(1)  No color(5,6,8,9)  Violet(7)
                                              |
                                          Strip Four
                                              |
                                              D
                                              |
                                              G
                                        ┌─────┴─────┐
                                  No color(6,8,9)   Red(5)
                                        |
                                    Strip Five
                                        |
                                        C
                                        |
                                        G
                                  ┌─────┴─────┐
                               Red(8)      No color(6,9)
                                              |
                                          Strip Six
                                              |
                                              B
                                              |
                                              C
                                              |
                                              G
                                        ┌─────┴─────┐
                                     Red(6)      No color(9)
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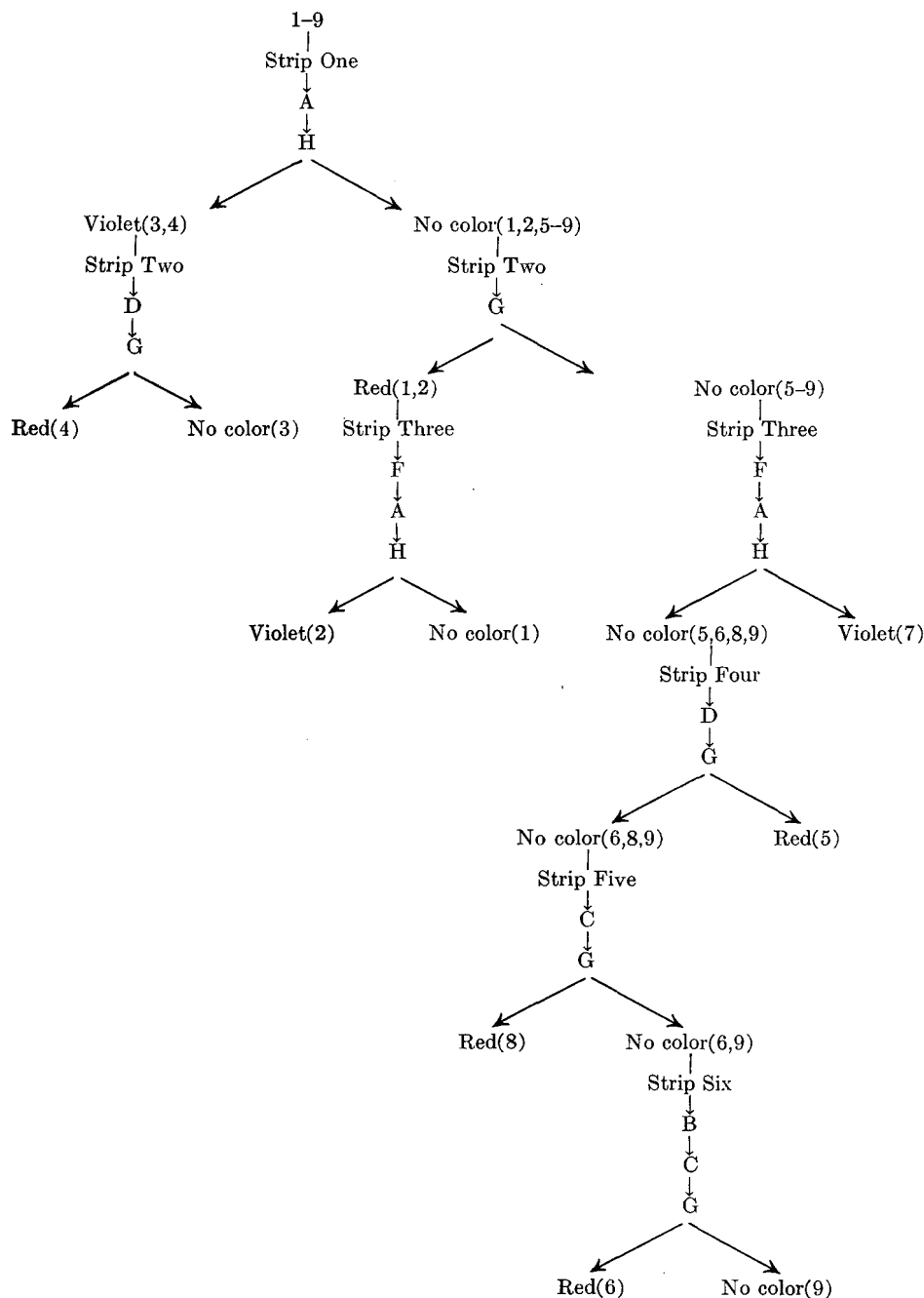
**Scheme I.   Scheme of analysis to determine the identity of side chains 1 to 9. Letters refer to reagents described in text.   Arabic numbers refer to side chains shown in Figure 1**

D.   Chromium trioxide–acetic anhydride oxidation is carried out by dipping the test paper into the reagent, placing it on a glass plate, and allowing most of the acetic anhydride to evaporate.   The plate is then transferred to the hot plate and the paper thoroughly dried.

E.   Oppenauer oxidation is effected by immersing the test strip in the reagent, placing it on the hot plate on a preheated glass plate, and covering it with another glass plate.   After 2 to 3 minutes, the top plate is removed and the paper thoroughly dried.

F.   The lithium aluminum hydride reagent is covered and allowed to boil, and the strip is then momentarily immersed until the vigorous bubbling ceases (about 7 to 10 seconds).   It is then immediately placed between two glass plates and heated for 2 minutes on the hot plate.   The top plate is removed and the strip dried.   The strip is then dipped into tap water until bubbling ceases (destruction of the lithium aluminum hydride), blotted

on its underside with filter paper, and returned to the hot plate until it is dry.

G.   The strip is dipped into the reagent, held for a few seconds, blotted on one side with filter paper, and allowed to develop on another piece of filter paper.

H.   This reagent is used exactly as previously described (3).

Reaction with either Reagents G or H is always the last step in any series of reactions on a test strip.

### RESULTS AND DISCUSSION

The above reagents used in proper sequence with 15-γ portions of an unknown steroid on paper will specifically identify all of the known side chains of adrenocortical and sex hormones.   The reactions of these reagents with each of the groups representing the steroid side chains are known (7, 9, 15, 20, 21) and the end products represent derivatives.

In general, the schemes of analysis depend upon the following organic reactions.

Of all the side chains in Figure 1, only 3 and 4 are oxidized to 17-ketones by periodic acid (Reagent A).

Chromic acid (Reagent B) will oxidize side chains 2, 3, 4, and 9 to 17-ketones, and side chain 6 to a methyl ketone (Scheme I, Strip 6).   The lead tetraacetate then oxidizes the methyl ketone to an α-ketol acetate which yields a red color with Reagent G.   Although it is known that chromic acid will oxidize side chain 7 to a 17-ketone, under the conditions of the test and using the reagent as described above, side chain 7 is not appreciably oxidized to a 17-ketone in useful quantities in these micro quantities for this test series.

Oxidation by lead tetraacetate (Reagent C) will produce a dihydroxy-acetone acetate and an α-ketol acetate from side chains 7 and 8, respectively.   All others are dissimilarly oxidized (if at all).

Chromium trioxide–acetic anhydride (Reagent D) oxidizes side chains 4 and 5 to dihydroxyacetone acetate and α-ketol acetate side chains, respectively.   Side chains 3 and 6 to 9 do not yield these required structures upon oxidation and subsequently do not react with Reagent G.   Side chains 1 and 2 are acetylated by this reagent but are otherwise unaltered.

Oppenauer oxidation (Reagent E) produces a 17-ketone only from side chain 9.

Lithium aluminum hydride (Reagent F) reduces the 20-ketone in side chains 1, 2, 7, and 8 to hydroxyl groups.   The reagent also reduces the 3-, 11-, and 17-ketones of steroids.

Triphenyltetrazolium chloride Reagent G is reduced by the α-ketol or dihydroxyacetone side chain. Used on a strip after Reagent C, a positive reaction has occurred if the spot turns pink-red after 6 to 8 minutes, usually against a very light pink background on the paper. The alkali in this reagent must first hydrolyze the acetate formed with Reagent C and then Reagent G reacts, and this accounts for the time lapse. Reaction with Reagent D on a test spot followed by Reagent G yields a reddish pink spot in 2 to 4 minutes which is always, in a positive test, more intense with side chain 5 than 4. The splitting of the acetate may also account for the time lag. No background color on the paper is found after this time. Immediate deep coloration occurs with side chains 1 and 2 as described by Burton and co-workers (5).

Although side chains 1 and 2 may be differentiated by a previous test (1), the method as described in Schemes I and II is very reliable in these microquantities and utilizes already available reagents for these tests.

Dinitrobenzene (Reagent H) reacts with all the 17-ketone derivatives (products of Reagents A and B) at about the same rate and to the same extent. One exception is the reaction between Reagent H and the product of Oppenauer oxidation. A positive reaction occurs and a brilliant violet ensues with only the slightest warming of the strip and in less than a minute. Some slight discoloration of the paper, coloring it in parts violet-purple, may also occur at this time.

Many schemes of analysis are possible in which the above organic reagents are compatible for successive reactions on a single test strip. Schemes I and II, however, represent two of the tried and successful combinations.

For paper chromatography, it is assumed that narrow test strips from a chromatogram have been tested with the modified Zimmermann dinitrobenzene reagent to rule out immediately the presence of a 17-ketone, before eluting the unknown compound for differential chemical testing.

Schemes I and II are very useful for determining the identity of the side chain of compounds after paper chromatography, since by chromatography the relative polarity of a compound (and therefore an indication of its side chain polarity) is known. By
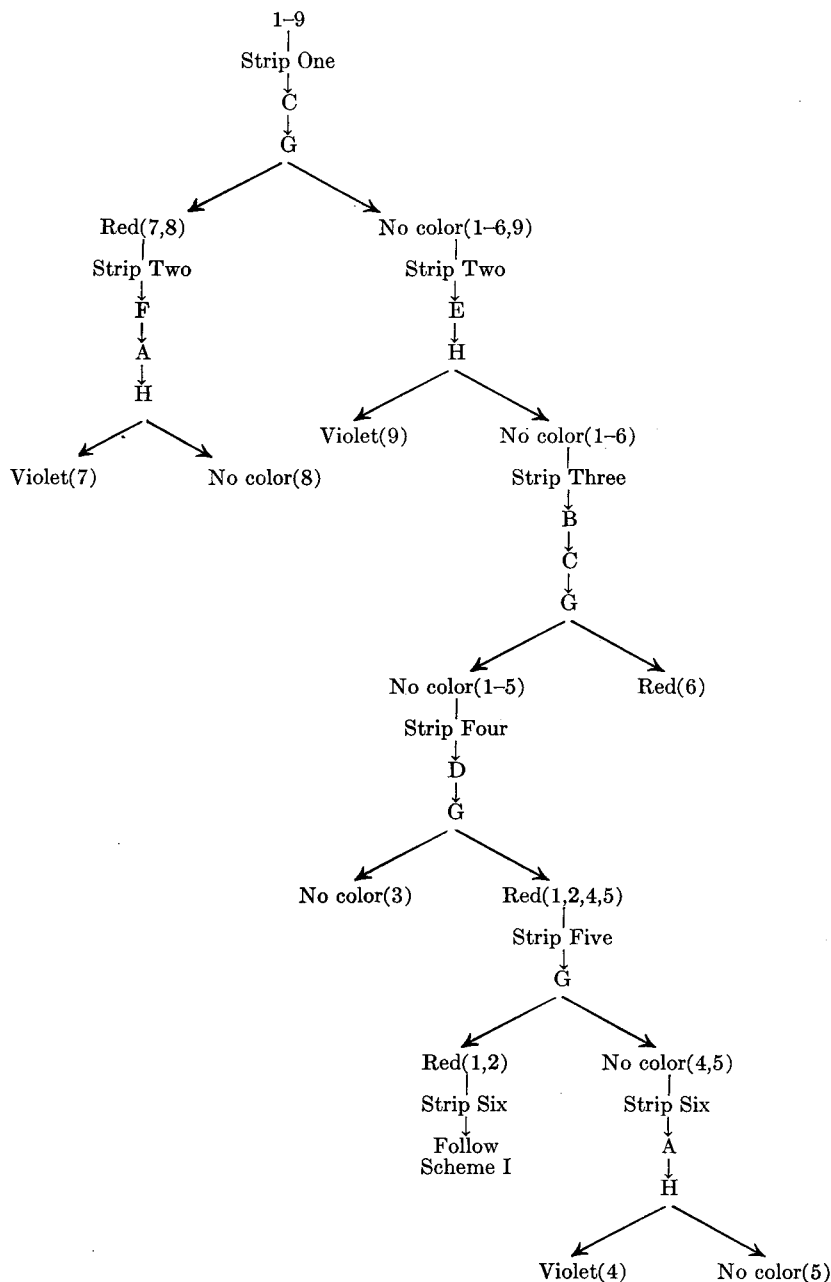


Scheme II. Scheme of analysis to determine the identity of side chains 1 to 9. Letters refer to reagents described in text. Arabic numbers refer to side chains shown in Figure 1

following Scheme I, the analysis of very polar side chains is performed with no more than 45 γ (three strips) whereas 60 to 90 γ (4 to 6 strips) are needed to analyze the least polar side chains. The reverse is true if Scheme II is followed, and the least polar side chains are identified with no more than 45 γ (three strips).

If the relative polarity of the compound is not known, such as when the side chain resulting from a synthetic reaction is to be determined, Schemes I or II or modifications of these may appropriately be applied for its analysis. No more than 60 γ (four strips) are required for the determination of any of the nine side chains in these schemes. Although test strips containing 15 γ each have been used uniformly throughout this investigation, many of the chemical reactions can be successively and

successfully executed with test strips which contain only 5 to 6 γ of starting compound in a spot 0.7 cm. in diameter. To conserve further the compound, the strip may be cut in half, thus halving the spot, and each half used is a different organic chemical reaction.

## ACKNOWLEDGMENT

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# Determination of Traces of Certain Rare Earths in Zirconium

## Ion Exchange Separation and Spectrographic Determination of Fractional Part-per-Million Amounts

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The regenerative efficiency and neutron economy of thermal reactors in which zirconium metal is used as a structural material may be reduced by the presence of fractional part-per-million amounts of certain rare earth elements as impurities in the zirconium. To determine these rare earths at such low concentrations, an analytical method has been developed which is based on ion exchange separation of zirconium as the fluozirconate anion, separation of the rare earths plus yttrium carrier from other nonrare earth impurities by classical chemical separations, and spectrographic determination of the individual rare earths (gadolinium, terbium, dysprosium, holmium, samarium) in the yttrium used as the carrier. Quantitative recoveries are obtained within the limits of experimental error of ±10%.

THE low thermal neutron cross section (0.18 barn), high melting point, good strength at moderately high temperatures, workability, and corrosion-resistance properties of zirconium make this metal a very useful structural material in thermal reactors (12, 13). The regenerative efficiency and neutron economy of thermal reactors can be reduced appreciably if trace amounts of elements possessing high thermal neutron cross sections are present as impurities in the zirconium metal. Several of the rare earth elements possess very high cross sections— 46,000, 8100, 4200, and 1150 barns, respectively, for gadolinium, samarium, europium, and dysprosium (17). It is therefore essential that these rare earth elements, especially gadolinium, not be present in zirconium in amounts greater than 1 p.p.m.

The sensitivities of detection of the most sensitive spectrographic (4-7, 15) or spectrophotometric (15, 18, 19) methods of analysis for these rare earths are in the 50- to 100-p.p.m. range. Consequently, prior concentration of the rare earths from the zirconium matrix is necessary to obtain the desired sensitivity.

Since zirconium metal is readily soluble only in hydrofluoric acid or acid mixtures containing hydrofluoric acid, and since the rare earth fluorides are normally quantitatively insoluble, it would seem that the concentration of rare earths would be a simple matter. In practice, however, the rare earth fluorides are not precipitated under the environmental conditions of this solution, even after the addition of as much as 20 mg. of a carrier rare earth. Consideration of the solubility product of the rare earth fluorides (estimated to be approximately $10^{-17}$), the ionization constant of hydrofluoric acid ($6.7 \times 10^{-4}$), the equilibrium constant for the formation of the bifluoride ion (3.8), the reduction of the activity coefficients by the high ionic strength of the solution (21), and the pH (~0.1) indicates that under these conditions the solubility products of the rare earth fluorides are not exceeded. However, in cases where an insoluble fluoride of some nonrare earth impurity in the zirconium is precipitated, this precipitate is observed to be contaminated with rare earth fluorides.

The fact that zirconium is present as the very stable fluozirconate anion whereas the rare earths are present as cations in these hydrofluoric acid solutions suggests the use of a cation exchange column separation. Thus, if this solution is passed through a cation exchange column, the rare earth cations undergo ion exchange and are retained while the fluozirconate anions pass through the column.

The procedure described in this paper is based on ion exchange separation of zirconium as the fluozirconate anion, removal from the concentrated rare earths of interfering nonrare earth impurities, and spectrographic determination of the individual rare earths in the yttrium oxide used as a carrier in the separation operations. Yttrium was chosen as the carrier because of its simple spectrum and the almost identical similarity of its chemical properties with those of the rare earths under investigation.

Since prior spectrographic calibrations were available for the determination of samarium, gadolinium, terbium, dysprosium, and holmium in an yttrium oxide matrix, holmium and terbium were included in this study, even though their neutron absorption cross sections are small. Because of the very low natural abundance of europium, a specific method for its determination was unnecessary.

## APPARATUS

The ion exchange column is designed to withstand the attack of both hydrofluoric and hydrochloric acids. The column consists of 1-inch inside diameter poly(vinyl chloride) (Tygon) tubing encased within a $1\frac{1}{4}$-inch inside diameter glass tube for support. The reservoir is a 1-liter polyethylene funnel. The 36-inch-long resin bed is supported on a plug of polyacrylonitrile (Orlon) filter cloth, kept in place by constricting the Tygon tube with hose clamps on each side of the plug. Dowex 50, 50- to 60-mesh (obtainable from the Dow Chemical Co.) is used as the ion exchange resin.

## CONCENTRATION AND PURIFICATION PROCEDURE

A flow sheet of the complete ion exchange concentration and chemical purification procedure is shown in Figure 1. A 100.0-gram sample of zirconium metal is placed in a 3-liter polyethylene beaker containing 1800 ml. of distilled water. To this are added 285 grams (240 ml.) of 48% hydrofluoric acid. After reaction has ceased, 20.0 mg. of 99.5% pure yttrium oxide dissolved in dilute hydrochloric acid are added to the solution. This yttrium oxide should preferably contain approximately 0.1% samarium, 0.05% gadolinium, 0.05% terbium, 0.05% dysprosium, and 0.05% holmium. The solution is allowed to cool, then diluted to 2000 ml., mixed well, and passed through the ion exchanger at a flow rate of about 10 ml. per minute. As the last few milliliters of solution pass into the resin bed, the reservoir is washed with 50 ml. of distilled water. This washing is repeated until the effluent tests neutral to pH paper. The column may then be eluted with 3 liters of 6$N$ hydrochloric acid.
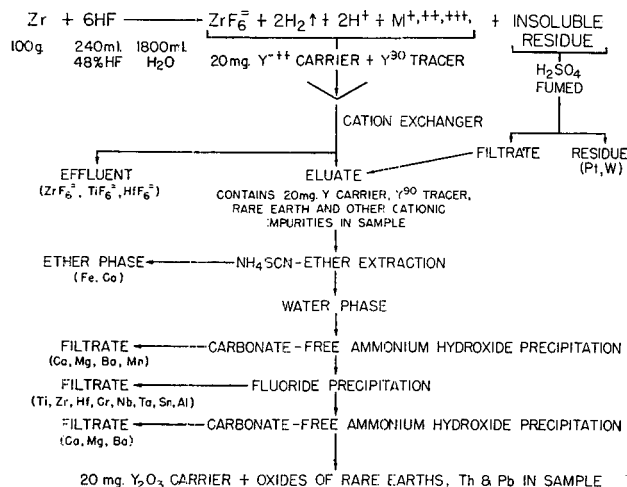


**Figure 1. Outline of concentration and purification procedure**

Since the capacity of an ion exchange column is limited, and since the amounts of common impurities present in the zirconium are usually unknown, a particularly impure sample of zirconium may saturate the column, whereupon break-through occurs. To guard against this, a solution of yttrium-90 tracer may be added (20). Periodic check of the effluent with a beta counter detects any break-through. This is probably unnecessary, since a 50% safety margin was provided in the column size and amount of eluant.

Any precipitate which forms after dissolution of the zirconium in hydrofluoric acid and subsequent addition of the yttrium carrier is filtered through S & S No. 589 white label paper. This filtration is performed immediately prior to passing the solution through the ion exchanger. This residue is ignited only long

## Table I. Operating Details for Analysis of Rare Earths

| Line pairs | | |
|---|---|---|
| $\dfrac{\text{Tb } 3218.43}{\text{Y } 3182.42}$ | 0.1 to 2.0% of $Tb_4O_7$ in $Y_2O_3$ | |
| $\dfrac{\text{Gd } 3350.48}{\text{Y } 3377.71}$ | 0.02 to 2.0% of $Gd_2O_3$ in $Y_2O_3$ | |
| $\dfrac{\text{Gd } 4327.10}{\text{Y } 4291.03}$ | 0.1 to 2.0% of $Gd_2O_3$ in $Y_2O_3$ | |
| $\dfrac{\text{Dy } 3407.80}{\text{Y } 3393.12}$ | 0.01 to 0.2% of $Dy_2O_3$ in $Y_2O_3$ | |
| $\dfrac{\text{Dy } 3407.80}{\text{Y } 3450.95}$ | 0.1 to 2.0% of $Dy_2O_3$ in $Y_2O_3$ | |
| $\dfrac{\text{Ho } 3453.14}{\text{Y } 3393.12}$ | 0.02 to 0.2% of $Ho_2O_3$ in $Y_2O_3$ | |
| $\dfrac{\text{Ho } 3453.14}{\text{Y } 3450.95}$ | 0.1 to 2.0% of $Ho_2O_3$ in $Y_2O_3$ | |
| $\dfrac{\text{Sm } 4334.14}{\text{Y } 4291.03}$ | 0.1 to 2.0% of $Sm_2O_3$ in $Y_2O_3$ | |

| | |
|---|---|
| Composition of sample charge | One-half of the yttrium–rare earth oxide concentrate, blended with 8 mg. of 200-mesh powdered graphite |
| Spectrograph | Jarrell-Ash, 3.4-meter stigmatic grating spectrograph |
| Upper electrode (cathode) | Graphite rod, $\frac{1}{8}$-inch diameter, pointed at one end |
| Lower electrode (anode) | Shallow thin-walled graphite electrode ($\frac{1}{4}$-inch diameter graphite with 2-mm. deep cavity and wall thickness of $\frac{1}{2}$ mm.). Anode is supported on $\frac{1}{8}$-inch graphite pedestal (4–7) |
| Analytical gap | 4 mm. |
| Excitation source | D.c. arc, 250 volts, 17–18 amperes (between clean graphite electrodes in air) |
| Length of exposure | Sample arced to complete consumption |
| Emulsion | Spectrum analysis No. 1 (3200–3800 A.) Kodak $M^a$ (3800–4400 A.) |
| Wave-length region | 3200–4400 A., second order |
| Filter | No. 7740 Corning |
| Slit | 0.040 mm. |
| Development | Four minutes at 21° C. in Eastman Kodak D-19 with continuous agitation |
| Densitometry | Applied Research Laboratories Comparator-Densitometer |
| Emulsion calibration | Two-step sector, preliminary curve method (3) |

$^a$ Kodak M plates should not be confused with class M spectral sensitivity. The Class B panchromatic sensitivity, high resolving power, and high contrast of Kodak M plates make them very useful plates for analytical spectroscopy in wave-length region between 4300 and 6700 A.

enough to destroy the filter paper, because prolonged ignition makes many oxides and fluorides difficult to redissolve. To convert any rare earth fluorides in the ignited residue into the soluble sulfates, 25 ml. of concentrated sulfuric acid are added and the mixture is fumed to dryness. The residue is leached with 50 ml. of 6$N$ hydrochloric acid for 1 hour and then is filtered. The filtrate is added to the eluate.

The eluate is boiled down to 15 ml. to concentrate the solution and to remove most of the hydrochloric acid. During this process, a precipitate sometimes appears which may be insoluble sulfates, hydrated silicic acid, or dissolved resin. If the precipitate does not dissolve after dilution to 250 ml. and 1-hour digestion, it may be filtered off and discarded.

The eluate contains not only the yttrium carrier and the rare earth impurities from the original sample, but also many other cations. The concentrations of the latter may be up to 1000 times greater than the rare earth concentration and hence may cause

**Table II. Analysis of Zirconium Samples after Addition of Known Amounts of Rare Earths**

| Impurity | Zirconium Sample | Y₂O₃ Carrier | Impurity Content of Y₂O₃ Carrier (Expressed as P.P.M. Impurity in Zr) Before concn. procedure | After concn. procedure | Normal Spectrographic Error |
|---|---|---|---|---|---|
| Gd | 1 | A | 0.043 | 0.046 | ±0.004 |
|    | 1 | B | 0.13  | 0.14  | ±0.015 |
|    | 2 | B | 0.13  | 0.14  | ±0.015 |
|    | 1 | C | 0.30  | 0.27  | ±0.03 |
|    | 2 | C | 0.30  | 0.29  | ±0.03 |
| Tb | 1 | A | n.d.ᵃ  | n.d. |  |
|    | 2 | A | n.d.  | n.d. |  |
|    | 1 | B | 0.51  | 0.51 | ±0.05 |
|    | 2 | B | 0.51  | 0.51 | ±0.05 |
|    | 1 | C | 1.07  | 1.00 | ±0.10 |
|    | 2 | C | 1.07  | 1.07 | ±0.10 |
| Ho | 1 | A | n.d.ᵇ  | n.d. |  |
|    | 2 | A | n.d.  | n.d. |  |
|    | 1 | B | 0.36  | 0.36 | ±0.035 |
|    | 2 | B | 0.36  | 0.39 | ±0.035 |
|    | 1 | C | 1.20  | 1.05 | ±0.11 |
|    | 2 | C | 1.20  | 1.16 | ±0.12 |
| Sm | 1 | A | n.d.ᶜ  | n.d. |  |
|    | 2 | A | n.d.  | n.d. |  |
|    | 1 | B | 0.16  | 0.16 | ±0.015 |
|    | 2 | B | 0.16  | 0.16 | ±0.015 |
|    | 1 | C | 0.74  | 0.66 | ±0.07 |
|    | 2 | C | 0.74  | 0.70 | ±0.07 |
| Dy | 1 | A | 0.013 | 0.012 | ±0.001 |
|    | 2 | A | 0.013 | 0.017 | ±0.001 |
|    | 1 | B | 0.42  | 0.44  | ±0.04 |
|    | 2 | B | 0.42  | 0.44  | ±0.04 |
|    | 1 | C | 0.71  | 0.63  | ±0.07 |
|    | 2 | C | 0.71  | 0.73  | ±0.07 |

ᵃ Not detectable is less than 0.04 p.p.m.
ᵇ Not detectable is less than 0.01 p.p.m.
ᶜ Not detectable is less than 0.1 p.p.m.

interferences in the spectrographic determination. For the determination of samarium, gadolinium, terbium, dysprosium, and holmium in yttrium, 11 analysis and internal standard lines are used. Elements which have lines within 0.2 A. of a rare earth line are classified as interferences. From wave-length tables (14) it was determined that small amounts of barium, calcium, chromium, cobalt, hafnium, iron, manganese, molybdenum, niobium, osmium, palladium, platinum, rhenium, rhodium, silver, strontium, tantalum, thorium, tin, titanium, tungsten, uranium, vanadium, and zirconium might cause interferences. To remove these interference uncertainties, the rare earth concentrate is given a purification treatment, whose essential steps are outlined in the flow sheet (Figure 1).

For optimum extraction of the iron as the thiocyanate complex, the solution should be at 0° C. and pH of 1 since the distribution coefficient, $C_{ether}/C_{water}$, decreases from 1.9 at 0° C. to 0.23 at 25° C. (8). Crushed ice made from distilled water may be added to the separatory funnel as needed. Successive 2-gram portions of ammonium thiocyanate are added to the solution. After each addition of thiocyanate, the solution is extracted with 200-ml. portions of ether until the violet ether layer becomes red. The final extraction should yield a very light pink ether layer.

The water phase is boiled to expel ether and carbon dioxide. After cooling to approximately 80° C., 5 drops of phenolphthalein indicator are added and ammonia gas is passed into the solution until the end point is reached. The ammonia gas is passed into a hot solution to approximate the conditions of homogeneous precipitation. A carbonate-free hydroxide precipitation at pH 9 quantitatively precipitates the rare earths while the more soluble hydroxides of magnesium and the alkaline earths remain in solution. The elements which form stable ammonia complexes are also removed. After digestion and cooling, the mixture is filtered through S & S No. 589 white or blue label paper.

After ignition to destroy the filter paper, the residue is treated with 50 ml. of 48% hydrofluoric acid and digested until the ignited oxides are dissolved, adding more hydrofluoric acid as required. The mixture is then diluted to 100 ml., cooled, and the yttrium, rare earth, and other insoluble fluorides separated by filtering through S & S No. 589 blue label paper using polyethylene funnels. The filtrate will contain the zirconium present in the original eluate. This zirconium may have been introduced into the eluate by insufficient washing of the column or by the sulfuric acid extract of the insoluble residue.

Then 25 ml. of concentrated sulfuric acid are added to the ignited fluorides and the mixture is fumed to dryness. The residue is dissolved in 100 ml. of 0.5N hydrochloric acid, 5 drops of

phenolphthalein indicator are added, and the rare earth hydroxides are precipitated with ammonia gas. If more than 25 mg. of ignited oxides are obtained, the fluoride and hydroxide precipitations are repeated; but if less than 25 mg. are obtained, the residue is analyzed spectrographically. Rhodium, thorium, lead, and thallium are not separated from the rare earths by the above procedure. Consequently, if insufficient decrease in residue weight is observed after repeating the fluoride and hydroxide precipitations, a spectrographic qualitative analysis should be performed on a portion of the concentrate to determine which of the above elements is the major impurity in the residue. Thorium may be removed by precipitation with hexamethylenetetraamine (9, 10). Lead may be removed by precipitation as the sulfate (2). Separations for rhodium and thallium were never found to be necessary in any of the analyses performed in this laboratory. Of the above elements only rhodium and thorium are interferences for the analysis lines. Since neither is a serious interference, concentrations of these elements up to 10% may be tolerated in the rare earth concentrate.

## SPECTROGRAPHIC DETERMINATION OF RARE EARTHS

The spectrographic determination of the gadolinium, samarium, terbium, dysprosium, and holmium contents of the yttrium oxide carrier is performed by a method originally developed for the determination of the respective rare earth impurities in pure yttrium oxide. The over-all procedure is patterned after the methods previously described (4–7) for the quantitative determination of other rare earth impurities in rare earths purified by F. H. Spedding and associates at the Ames Laboratory. The method is based on the direct current carbon arc excitation of yttrium oxide–graphite mixtures. Selected yttrium lines are used as internal standards. Pertinent experimental details of the procedure are summarized in Table I. Photographic processing, photometry, and intensity ratio determinations follow standard practices (16). The line pairs employed for the determinations are summarized in Table I.

## RESULTS

As no other analytical methods were available to check the accuracy of the zirconium analyses, a series of recovery experiments was performed. Three hundred grams of two different reactor grade zirconium metal samples (labeled as 1 and 2) were dissolved, and each solution was divided into three aliquots. Twenty-milligram portions of three yttrium oxide samples labeled as A, B, and C containing known, but different, amounts of samarium, gadolinium, terbium, dysprosium, and holmium were added to each of the three aliquots. Column 4 of Table II presents the analyses of the three yttrium oxide samples. The values, expressed as parts per million of impurity metal in zirconium metal, represent the concentrations of these impurities which were added to the zirconium aliquots before their analysis. After processing, the three yttrium oxide residues were analyzed. The difference in value between analyses of the original oxide and the oxide isolated as the carrier is a measure of the amount of rare earth impurity present in the zirconium. Within normal spectrographic error, this difference should be the same for each of the three yttrium oxide carriers added to the same zirconium sample. Column 6 lists the variation attributable to normal spectrographic error for each range, which is about ±5% of the amount present. Since in the performance of the recovery experiment it was necessary to analyze the original as well as the treated yttrium oxide, the values in columns 4 and 5 are both subject to ±5% error, so that the errors observed in this case may be as much as ±10%.

The results shown in Table II clearly indicate that the individual rare earth content of the two zirconium metal samples was less than 0.05 p.p.m. for the rare earths studied in this investigation. The results also clearly show that complete recovery was obtained for all of the rare earths under investigation. The sensitivity of the method may be increased by using an yttrium oxide carrier containing the smallest determinable amounts of each of the individual rare earths. In this manner, the

inertia of the emulsion is overcome by the amount originally present in the carrier, whereupon any small enrichment added to the carrier by the rare earth content of the sample is more readily apparent.

**Table III.  Selection of Range by Variation of Parameters**

| Zr Sample Size, G. | Wt. of Y$_2$O$_3$, Mg. | Range, P.P.M. |
|---|---|---|
| 500 | 10 | 0.003 to 0.3 |
| 100 | 20 | 0.03 to 3.0 |
| 1 | 40 | 6.0 to 600 |

The results obtained from the spectrographic analysis are in the form of per cent impurity oxide in yttrium oxide. These values may be calculated back to parts per million of impurity metal in zirconium metal as indicated in Equation 1 for gadolinium. The range over which ($X$) may vary is 0.02 to 2.0 mg., which is equivalent to 0.02 to 2.0% gadolinium oxide in the yttrium oxide matrix. Correspondingly, ($Z$) would vary from 0.03 to 3.0 p.p.m. However, this range may be changed by varying either the amount of the yttrium oxide carrier or the size of the zirconium sample. In Table III, proper choice of these parameters extends the range of analysis, for gadolinium, from 0.003 to 600 p.p.m. with equal accuracy.

$$\frac{\dfrac{(X) \text{ mg. Gd}_2\text{O}_3}{100 \text{ mg. Y}_2\text{O}_3} \times 20 \text{ mg. Y}_2\text{O}_3 \times \dfrac{314 \text{ mg. Gd}}{362 \text{ mg. Gd}_2\text{O}_3} \times \dfrac{10^{-3} \text{ g.}}{\text{mg.}}}{100 \text{ g. Zr}} \times$$

$$10^6 = (Z) \text{ p.p.m. } \frac{\text{Gd}}{\text{Zr}} \quad (1)$$

### DISCUSSION OF ERRORS

The terminology used in this discussion follows the conventions adopted by Kolthoff and Sandell (11).

Inherent in the concentration and purification procedure is a feature which practically eliminates the possibility of determinate errors—namely, that the yttrium oxide added as a carrier also serves as the internal standard for the spectrographic analysis. Once a homogeneous solution has been formed, the ratio of rare earth impurities to yttrium is established and remains constant, since no fractionation of the rare earth group occurs during the subsequent treatments. Consequently, loss of sample into the ether layer during extraction, or by improper or incomplete precipitation, or even by accidental spilling, does not affect the determination. Ten milligrams of yttrium oxide are required to perform the spectrographic analysis, but since 20 mg. are available, as much as half of the sample may be lost without invalidating the results.

The major portion of the experimental error in this method arises from the spectrographic determination. Photometry errors rarely exceed 2%, whereas the indeterminate errors caused by fluctuations in the direct current arc source may introduce 3 to 5% average deviation. These two errors comprise the 5% normal spectrographic error referred to earlier. However, spectrographic determinations are also subject to a much more unpredictable error known as the extraneous element effect (1). The rare earth concentrate obtained from the purification procedures may contain as high as 20% total of nonrare earth elements, which were either incompletely separated during the operations, or not separated at all in the case of lead, thorium, thallium, or rhodium. Since the final composition of the rare earth concentrate may vary from sample to sample, the effect of extraneous elements on the analytical intensity ratios was determined. Oxides of sodium, magnesium, strontium, aluminum, copper, lead, chromium, niobium, thorium, silicon, vanadium, and zirconium were added to yttrium oxide in the ratio of 80% of yttrium oxide and 20% of the extraneous oxide. These oxides were chosen to cover uniformly the boiling point range for the

metal oxides commonly encountered in spectrographic analyses. Since some of these elements have lines which interfere with one or more of the analysis lines of the rare earths, their effects were studied only on those analysis line pairs with which they did not interfere. Sodium, strontium, aluminum, chromium, niobium, silicon, and zirconium produced more than 10% error in some intensity ratios when present as 20% of the sample, but none of the elements tested produced more than a 20% error. Since none of these elements were ever found in the rare earth concentrate in amounts greater than a few per cent, little effect from them is expected in normal analyses. Therefore, errors smaller than 5% are not expected, and errors greater than 10% are not probable, so that assigning a value of ±10% error to this method is realistic.

### OTHER APPLICATIONS

The general procedure should be equally applicable to the determination of these 5 rare earths in any material which, upon treatment with hydrofluoric acid, dissolves to form a stable anionic complex. Among these may be mentioned beryllium, boron, silicon, germanium, titanium, hafnium, and probably vanadium. The purification procedure removes most other elements in addition to those designated as interferences in this method.

The ion exchange procedure described should prove useful also for the separation or concentration of any element which forms cations in hydrofluoric acid solution from those elements which form stable anionic complexes in hydrofluoric acid solution

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# Organic Spot Test Analysis

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**The feasibility of using organic preparation procedures as the bases of microanalytical tests for organic compounds or functional groups has been discussed and explored. Procedures for the detection of phenols, acyl derivatives of aromatic amines, arylurethanes and monoarylureas, and sulfonic acids have been developed on such foundations. The Vieboeck-Zeisel procedure for the quantitative determination of methoxy and ethoxy groups has been made the foundation of a sensitive test for organically bound iodine.**

EVEN though qualitative organic analysis at times employs methods based on procedures for preparing organic compounds, there has been little consideration of the general adapting of such procedures to analytical work. This neglect is not due solely to the admittedly wide differences between the functions and interests of preparative and analytical chemistry; there are other cogent reasons. In the first place, it is difficult to make a choice among the great number of preparation procedures, many of them involving intermediate stages. Manuals which stress the preparation of organic materials on a small scale have but recently become available—e.g. (*11*)—in the main it is difficult to find suitable working directions because the usual procedures employ amounts of the starting substances that are far greater than those used in macroanalysis. Furthermore, many procedures use nonionic reactions in nonaqueous media and these usually proceed slowly and often give low yields because of the intervention of side reactions. Moreover, the apparatus needed is frequently elaborate and the consumption of material is relatively high. Accordingly, at first glance preparative procedures appear to offer slight chance of success as a field for the discovery or development of new tests for organic compounds or functional groups, especially when semimicro or microanalytical goals are taken into consideration.

Recently it has been pointed out (*2, 3*) that the experience gained in the search for organic reagents and their development for use in inorganic analysis provides a new approach to the problem of making analytical employment of the reactions of organic compounds. Numerous organic reagents, whose use has facilitated the solution of difficult analytical problems (*18*), have been discovered by taking due account of the rule that the analytical action of organic compounds always rests on the presence and steric arrangement of certain salt-forming and coordinatable groups (*2*). The great importance of maintaining certain reaction conditions to secure maximum reliability and sensitivity has been thoroughly appreciated in many extensive studies dealing with organic reagents, and many instructive insights into the *modus operandi* of these reagents have been secured in this manner. As was to be expected, it was found that the organic compounds which function as sensitive reagents for certain ions can, in turn, be satisfactorily detected by means of these ions. However, these latter procedures almost never take the form of specific tests for the particular organic compound. Instead, the tests are directed toward the detection of the presence of characteristic groups in these compounds—i.e., of the "true functional groups." Pertinent instances are the tests for 1,2-dioximes, acyloinoximes, benzoinoximes, and sulfinic acids. Here there is a direct connection with qualitative organic analysis in which the detection of particular groups is of the highest importance.

Proceeding from this dual analytical employment of such in-organic-organic reactions, a further step can be taken—the preparation of organic reagents may be expected to make possible a test for the organic compounds involved in such preparative procedures. In other words, the sample being studied needs to be subjected to the conditions of the preparation of a particular organic reagent, which in turn can be identified by means of inorganic ions. Here again there is an obvious close relation to analytical research directed toward the discovery of organic reagents for inorganic ions. Of course, the analytical employment of preparative procedures must not be limited to the production of organic reagents; the goal must be more general. An analytical utilizability exists whenever the main product of a synthesis or preparation reaction can be detected or identified by means of inorganic or organic materials, or whenever this product is characterized per se by such things as its color, fluorescence, and solubility. In certain cases the detection of a characteristic side product may serve as a direct or indirect proof of the presence of an organic participant in the preparation process. Such cases require the recognition of the stoichiometric reaction underlying the preparation procedure. It is clear that the attainable yield of the particular organic product is no longer the most important factor. Instead, the usefulness of the procedure depends solely on whether certain products of the synthesis or preparative procedure are formed rapidly and in amounts sufficient to exceed the identification limits of their direct or indirect detectability. Consequently, in such cases, the statements regarding low yields lose their deterrent effect and several interesting aspects come into view.

In the first place, it may be expected that preparation procedures conducted on a small scale can still yield enough of the product to make its detection possible by macro-, semimicro-, and microanalytical procedures. Furthermore, attempts can be made to depart from rigid adherence to complicated working directions in order to achieve simplifications that are in line with analytical techniques. Such modifications might include changes of concentration and amounts of the reactants, shortening of the reaction period, and shifting of the scene of the reaction into the vapor phase. The emancipation from rigid procedures may even go much further if it is kept in mind that the sole consideration here is to detect an organic compound through its participation in a particular reaction. Consequently, it is permissible to go back to statements in the literature which report the formation of certain compounds but which have no real value from the preparation standpoint because the yields are inadequate. This category includes such methods as thermal and chemical fission processes, exchange of groups, fusion and sintering reactions, and the like. However, if such methods of forming the desired compounds go rapidly, if they can be translated into the chemical analytical technique, and if small amounts of the resulting product can be reliably detected by a simple test, then a better analytical utilization can be secured in this novel fashion than by a "tried and true" method of preparation which fails to meet adequately the conditions just cited.

Even though modification of the customary procedure usually leads to a lower yield, this disadvantage can be overcome in large part when analytical goals are being sought. Ordinary preparation procedures frequently necessitate the isolation of intermediate products and the purification of the final product. Both of these steps entail unavoidable losses. However, such losses are not encountered or need not be considered if the test can be

conducted directly on the reaction mixture without isolation of the intermediate products or removal of side products. This is often possible.

The foregoing considerations, which are based throughout on the chemistry of specific, selective, and sensitive reactions (2), have led to a series of studies which demonstrate that analytical application can be made of a number of familiar methods of synthesis. Their translation into the technique of spot test analysis has been found effective, since advantage may be taken of the many possibilities for enhancing the sensitivity of the particular test. Excellent instances of such classical syntheses are (4): the Reimer-Tiemann synthesis for o-hydroxyaldehydes (1875), the Skraup synthesis for quinoline (1882), and the Skinner-Ruhemann synthesis of diphenylcarbazide (1881).

The tests described in this paper further illustrate the fact that rich prospects are in store for utilizing the procedures of organic synthesis as well as hitherto neglected methods of forming organic compounds in the solution of problems presented by organic qualitative analysis. An example is also given to show that a quantitative method of organic analysis can be used as the origin of a qualitative test of wider applicability. Here too, valuable orienting ideas were drawn from the chemistry of specific, selective and sensitive reactions.

### DETECTION OF PHENOLS

In 1885, Illinsky and Knorre (7) made a study that has become one of the classics in analytical and coordination chemistry. They found that 1-nitroso-2-naphthol reacts with cobalt salts to yield a red-brown precipitate which dissolves in organic liquids Since then, a series of nitroso compounds with an analogous constitution has been recommended for the detection and quantitative determination of cobalt (19). In these reactions the o-nitrosophenol group (I) in its isomeric oxime form (II) yields chelate compounds with trivalent (in part also bivalent) cobalt (9)



(I)            (II)            (III)
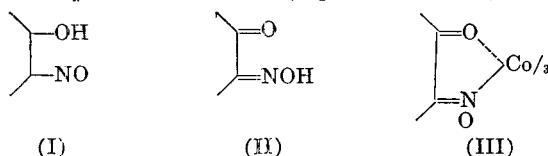
Compounds containing the chelate ring (III) are not soluble in water but do dissolve in solvents such as ether and chloroform provided the molecule contains no hydrophilic sulfonic or hydroxyl groups. Otherwise, the product is not soluble in organic liquids and dissolves in water because the chelate ring forms part of inner complex anions.

Compounds containing the group (I) are not only reagents for cobalt but in reverse they may be detected by means of cobalt ions through the production of colored chelate compounds. When conducted as a spot reaction on a porcelain plate, the identification limits are: 1 γ of 1-nitroso-2-naphthol, 1 γ of 2-nitroso-1-naphthol, 2.5 γ of nitroso-R salt, and 2 γ of dinitrosoresorcinol.

The spot test may be carried out on paper if desired. One drop of the test solution and 1 drop of cobalt(II) chloride solution are brought together and then held over strong ammonium hydroxide. This procedure is suggested when acid solutions are being examined.

The formation of cobalt-chelate compounds containing the group (III) can be used to detect those phenols which contain a free position ortho to the OH group. The phenol must be nitrosated in glacial acetic acid and the isolated nitroso compound treated with a cobalt salt in an acetate buffered solution. This cumbersome procedure is not really necessary. Phenols (solid or in solution) produce the colored cobalt-chelate compound directly if warmed with a water–acetic acid solution of sodium cobaltinitrite. This procedure provides the most favorable conditions for the production of these cobalt chelate compounds because the following partial reactions occur:

$$[Co(NO_2)_6]^{---} + 6H^+ \rightarrow Co^{+++} + 6HNO_2 \dots \quad (1)$$



$$\quad (2)$$



$$\quad (3)$$
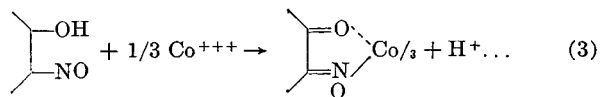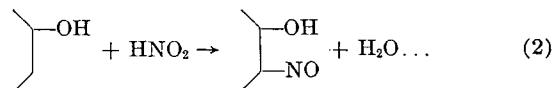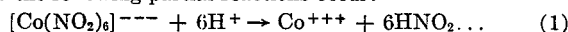
Reactions 1 to 2 and the associated chelation with a cobalt atom apparently favor the o-nitrosation of phenols. This is shown by the observation that phenol, which yields p-nitrosophenol almost exclusively on treatment with nitrous acid, produces considerable amounts of the cobalt salt of o-nitrosophenol if treated with an acetic acid solution of sodium cobaltinitrite.

The procedure described reveals the phenol character of compounds of rather complicated structure which do not respond to other phenol tests such as iron(III) chloride and the well known Liebermann reaction.

**Procedure.** One drop of the test solution or a grain of the solid is placed in a micro test tube and treated with a drop of freshly prepared (5%) water solution of cobaltinitrite and 1 drop of glacial acetic acid. A blank is set up at the same time; it contains a drop of water in place of the test solution. The two test tubes are heated over a free flame until the blank has become pink. A positive result with the sample is indicated by the production of a brown to yellow color or a brown precipitate. The latter is soluble in chloroform.

**Results.** This procedure reveals: 1 γ of 1-naphthol, 20 γ of sulfosalicylic acid, 5 γ of ellagic acid, 0.5 γ of morin, 5 γ of chromotropic acid, 5 γ of 1,4-naphtholsulfonic acid, and 0.5 γ of resorcinol.

A positive response was given by: 2-naphthol, pyrogallol, hydroquinone, pyrocatecholdisulfonic acid, p-hydroxydiphenyl, o-hydroxydiphenyl, adrenaline, gallic acid, tropaeolin O, morphine, thymol, arbutin, 2,4-dihydroxybenzaldehyde, o-hydroxyacetophenone, stovarsol (acetarsone), and eugenol. No reactivity was stated with o(m,p) hydroxybenzaldehyde or with salicylic acid.

When this test is used, primary aromatic amines should be absent if they exchange the $NH_2$-group for OH under the reaction conditions and hence falsely give the phenol reaction. If necessary, the amines can be removed prior to the test by extracting the alkaline solution with ether.

### DETECTION OF ACYL DERIVATIVES OF AROMATIC AMINES, ARYLURETHANES, AND MONOARYLUREAS

Bamberger (1) found, in 1894, that the solutions obtained after alkaline saponification of nitrosoacetanilide show the familiar color reaction of diazo compounds with naphthols and naphthylamines. He accordingly concluded that the reaction could be represented:
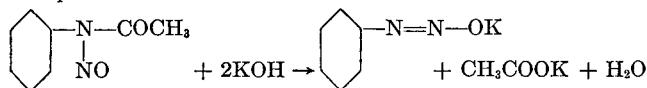


This behavior conforms to the assumption of an equilibrium between nitrosoacetanilide and the isomeric diazo acetate:
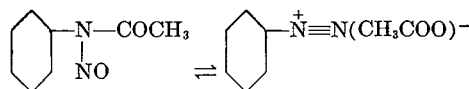


which on alkalization yields the alkali diazoate necessary to the coupling.

The conversion of nitrosoacetanilide into the diazoate, which is capable of coupling, was recommended by Rosenthaler (14) for the detection of acetanilide (and phenacetin) many years after Bamberger's original observation. The procedure is to mix a solution of 0.15 gram of acetanilide in 2 ml. of glacial acetic acid with 0.2 gram of sodium nitrite. After 10 minutes the solution is diluted with 3 ml. of water, made basic, and filtered. Three milliliters of the filtrate is diluted with 5 ml. of water and

treated with 0.1 gram of l-naphthol. A blood-red (orange) solution results.
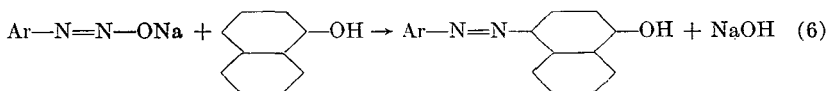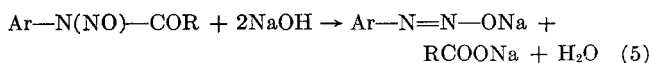
This procedure is based on the well known Fischer method (5) for the preparation of nitrosamines by nitrosation in glacial acetic acid. It suffices for the detection of acetanilide (phenacetin), but it does not meet the wider demands of the analyst. The ready nitrosability of phenols demonstrated by the above test and the sensitivity of the color reactions of diazo compounds led to the hope that the test would be capable of rapid execution under simpler conditions and with far less material. The following procedure, which employs spot test techniques, is very satisfactory, and it will reveal as little as 0.5 gamma of acetanilide.

**Procedure.** One drop of the test solution is treated in a micro test tube with 1 drop of 10% sodium nitrite solution and a drop of 1 to 1 hydrochloric acid. After 30 to 60 seconds a drop of 10$N$ sodium hydroxide is added. A pinch (tip of knife blade) of solid urea is added and the reaction mixture shaken. One drop of a 0.1% alcohol solution of 1-naphthol is introduced. On warming, a red or orange color appears, the shade depending on the quantity of acetanilide present.

A spot plate may also be used. A blank test is advisable only when very small quantities are being sought. If a solution of sodium nitrite is acidified with hydrochloric acid and then made alkaline, it gives a yellow color on the addition of 1-naphthol. This color reaction results from the formation of considerable amounts of nitrosyl chloride when sodium nitrite is treated with hydrochloric acid, and on alkalization there is consequent formation of alkali hypochlorite. The latter oxidizes 1-naphthol to a colored quinone. This interference may be avoided by adding urea. Alkaline solutions of $N$-nitroso compounds are not affected by urea.

It was logical to expect that the conversion of aromatic nitrosamines to diazo compounds is not limited to acetanilide and phenacetin (p-acetophenetidide, p-ethoxyacetanilide). Acyl derivatives of secondary aromatic amines should likewise undergo analogous reactions. Trials with a number of materials of the general structure Ar—NH—COR have shown that after nitrosation and subsequent alkalization there is always a coupling with 1-naphthol to produce an azo dye. (In the general formula, Ar—NH denotes the radical of a simple or condensed aromatic amine, and R denotes hydrogen, an alkyl or aryl group, or $OC_2H_5$ or $NH_2$.) Hence the realization of the following successive reactions permits the detection of acylides, arylurethanes, and monoarylureas:

$$Ar—NH—COR + HNO_2 \rightarrow Ar—N(NO)—COR + H_2O \quad (4)$$

$$Ar—N(NO)—COR + 2NaOH \rightarrow Ar—N=N—ONa +$$
$$RCOONa + H_2O \quad (5)$$



**Results.** The spot test procedure described yields the following limits of identification: 0.5 $\gamma$ of acetanilide, 0.5 $\gamma$ of acetylphenetidine, 1 $\gamma$ of p-acetylaminophenol, 5 $\gamma$ of 3-ureido-isonicotinic acid, 0.1 $\gamma$ of monophenylurea, 3 $\gamma$ of phenylurethane, 1 $\gamma$ of acetylarsanilic acid, and 1 $\gamma$ of acetylsulfathiazole. A positive response was given by acetylnaphthalide, stovarsol, and mono toluylurea.

When testing for the diazoates produced from $N$-nitroso compounds produced by Reactions 4 and 5 it is not permissible to replace Reaction 6 by the more sensitive coupling with 1-naphthylamine because the latter is diazotized by the excess nitrous acid and then couples to produce a foreign azo dye.

Attempts to remove the nitrous acid remaining after Reaction 4 by adding sodium azide showed that the nitroso compound was likewise destroyed—for instance, no color reaction with 1-naphthol ensued if the acidic solution was treated with an excess of sodium azide and then made basic. The azide or hydrazoic acid not only removes the nitrous acid (15)

$$HNO_2 + HN_3 \rightarrow H_2O + N_2O + N_2 \quad (7)$$
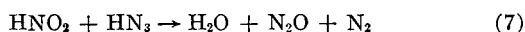
but obviously also destroys the nitrosamine with regeneration of the starting compound:

$$Ar—N—COR + HN_3 \rightarrow Ar—NH—COR + N_2O + N_2 \quad (8)$$
$$\underset{NO}{|}$$
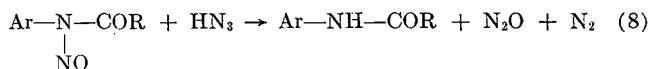
It was found that $N$-nitrosodiphenylamine, as well as other nitrosamines, are similarly decomposed by hydrazoic acid with regeneration of the respective secondary amines, whereas the $C$-nitroso compounds remain unaltered even when warmed with sodium azide. These facts were established by testing: p-nitrosodiphenylamine, nitrosoantipyrine, p-nitrosodimethyl- and diethylaniline, 1- and 2-nitrosonaphthol, and nitroso-R salt. Since the Liebermann indophenol reaction is given by both $C$- and $N$-nitroso compounds, prior treatment with hydrazoic acid may well make possible a very desired differentiation between these classes of nitroso compounds. It is likely that the decomposition with hydrozoic acid may be made the basis of a new gasometric method of determining $N$-nitroso compounds. It would probably be simpler and more accurate than the present gas-volumetric Lehmstedt procedure (10) which is based on measurement of the volume of nitric oxide evolved by the action of hydrochloric acid–iron(II) chloride solution, and which is applicable to both $C$- and $N$-nitroso compounds.

### DETECTION OF SULFONIC ACIDS

It was shown in 1870 by Meyer (13) that when alkali salts of benzenesulfonic or 1-naphthalenesulfonic acid are fused with sodium formate, the alkali salts of the respective carboxylic acids are formed. Meyer was of the opinion that this exchange of the sulfonic acid group for the carboxyl group might serve for the production of aromatic carboxylic acids. However, there is no record of the use of this reaction:

$$RSO_3K + HCOONa \rightarrow RCOONa + KHSO_3$$
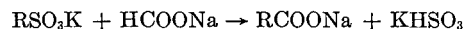
The procedure has no preparative significance probably because of the unavoidable thermal decomposition of the alkali salts of carboxylic acids formed. The reaction is cited, if at all, only for the formation of benzoic acid (8).

However, from the analytical standpoint, it might have been expected that this reaction could serve as the basis for an indirect test for aliphatic and aromatic sulfonic acids through detection of the resulting sulfite. Experiments along this line have demonstrated that even slight amounts of sulfonic acids yield alkali sulfite when evaporated with alkaline solutions of sodium formate and the evaporation residue then heated briefly. The presence of sulfite is readily revealed by the usual tests.

When alkali salts of sulfonic acids are fused with an excess of sodium formate, the reoxidation of the resulting sulfite to sulfate is prevented by the thermal decomposition:

$$2HCOONa \rightarrow 2H + (COONa)_2 \text{ and}$$
$$(COONa)_2 \rightarrow Na_2CO_3 + 4CO$$
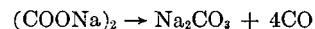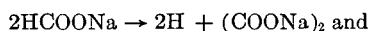
To detect the sulfite it is best to use the test devised by Heisig and Lerner (6), in which the volatile sulfurous acid liberated by mineral acids is allowed to react with ferri-ferricyanide. Prussian blue results.

**Reagents.** Alkaline sodium formate solution, 5 grams of sodium formate and 6 grams of sodium hydroxide in 100 ml. of water.

Ferri-ferricyanide solution, 0.08 gram of hydrated iron(III) chloride and 0.1 gram of potassium ferricyanide in 100 ml. of water plus sulfuric acid.

**Procedure.** The usual apparatus (4), designed to capture gases in hanging drops of reagent solution, is used. A little of the solid being examined is placed in the bulb of the apparatus along with a drop of the alkaline formate solution. If preferred, a drop of the water test solution may be taken for the test. After the mixture has been evaporated to dryness, the residue in the bulb is heated over a free flame until a gray color indicates incipient charring. This step ordinarily requires not more than 30 to 60 seconds. After cooling, the mass is acidified with sulfuric

acid and the knob of the stopper is charged with a drop of ferri-ferricyanide solution. The apparatus is closed and the hanging drop observed. If it turns blue, the presence of a sulfonic acid is indicated. Even slight colors are made visible if the drop is transferred to a white porcelain plate for examination.

**Results.** The procedure reveals the following in one drop of test solution: 2.5 $\gamma$ of naphtholsulfonic acid (1,4; 1,5; 2,6), 2.5 $\gamma$ of naphtholdisulfonic acid (2,6,8), 1 $\gamma$ of dihydroxynaphthalendisulfonic acid (1,8,3,6), 5 $\gamma$ of dihydroxynaphthalensulfonic acid (2,3,6), 2.5 $\gamma$ of naphthylaminesulfonic acid (1,3; 2,6), 1 $\gamma$ of naphthylaminedisulfonic acid (2,3,6), 1 $\gamma$ of sulfanilic acid (p-aminobenzenesulfonic acid), and 0.25 $\gamma$ of taurine (2-amino-ethanesulfonic acid). Positive response was given by H acid, sulfosalicylic acid, Congo red, tartrazine, sulfapyridine, sulfamethazine, sulfonal, and trional.

### DETECTION OF ORGANICALLY BOUND IODINE

Vieboeck and his associates described (16, 17) an elegant method for determining methoxy and ethoxy groups. It is based on the Zeisel method (20) of warming the sample with concentrated hydriodic acid; the resulting volatile alkyl halide (boiling point of methyl iodide, 45° C.; boiling point of ethyl iodide 73° C.) is transferred by means of carbon dioxide into a glacial acetic acid solution of bromine and sodium acetate:

$$C_2H_5I + Br_2 \rightarrow C_2H_5I \cdot Br_2 \qquad (9)$$
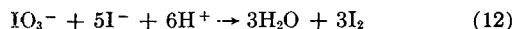
$$C_2H_5I \cdot Br_2 \rightarrow C_2H_5Br + IBr \qquad (10)$$

$$IBr + 2Br_2 + 3H_2O \rightarrow HIO_3 + 5HBr \qquad (11$$
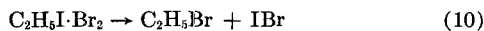
The resulting iodic acid is freed of excess bromine by adding formic acid and then determined iodometrically on the basis of the reaction:

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3H_2O + 3I_2 \qquad (12)$$

Since one molecule of methyl (ethyl)iodide is thus caused to yield six equivalents of titratable iodine, the Vieboeck method is very accurate and can be employed microanalytically. It has the added advantage of being applicable directly to sulfur-bearing compounds, which is not true of the original Zeisel method (12).

If it is assumed that the primary necessary reaction between bromine and the alkyl iodide produces an equilibrium

$$2RI + Br_2 \rightleftharpoons 2RBr + I_2 \qquad (13)$$
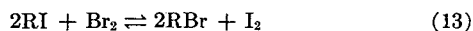
then the quantitative completion of the displacement reaction is ascribable to the removal from the equilibrium of the products shown on the right side of the equilibrium expression. The volatility of the methyl bromide (boiling point, 4.5° C.) can be a factor here, but the same can hardly be said of ethyl bromide (boiling point, 38.4° C.). However, as the removal of one of the involved materials is sufficient to cause an equilibrium reaction to go to completion (provided the equilibrium is re-established at an adequate speed), it is permissible to assume that the decisive factor in the Vieboeck procedure is the removal of iodine from Reaction 13 through conversion into iodic acid:

$$I^- + 5Br^- + 3H_2O \rightarrow HIO_3 + 5HBr$$
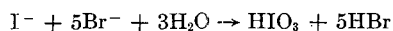
From this standpoint it may be expected that all organic iodine compounds which are capable of entering into the reversible Reaction 13 must behave similarly to ethyl and methyl iodide. As it is easy by Reaction 12, to detect the production of iodic acid, the correctness of the ideas just advanced should lead to a sensitive test for iodine bound to carbon. Trials have shown that this is actually the case.

To bring Reaction 13 about it is sufficient to employ bromine water, or a solution of bromine in potassium bromide which provides a higher concentration of bromine. The excess bromine can be removed instantly after the sample has reacted with the free halogen by adding solid sulfosalicylic acid, which forms colorless bromosulfosalicylic acid. This step also provides, without diluting the mixture, the acidity required for Reaction 12. The

iodine liberated can be detected by adding starch or by extraction with chloroform. The use of Thyodene indicator, which gives a blue color with traces of iodine, has proved excellent in this procedure.

**Reagents.** Saturated solution of bromine in 5% potassium bromide solution. A 5% solution of potassium iodide containing 5% Thyodene indicator.

**Procedure.** The test is conducted in a micro test tube. A drop of the test solution or a pinch of the solid being examined is treated with 1 drop of the bromine solution. The mixture is warmed gently and then allowed to cool. Solid sulfosalicylic acid is added gradually until the color is completely discharged. A drop of water is added, and the liquid is shaken to remove any bromine from the vapor above the solution. A drop of potassium iodide solution is then introduced. A blue color appears, the intensity depending on the quantity of iodine in the sample. When very dilute solutions of iodine are being tested, it is advisable to conduct a comparison blank.

**Results.** This procedure reveals 0.05 $\gamma$ of methyl iodide, 0.1 $\gamma$ of iodoform, 0.05 $\gamma$ of 7-iodo-8-hydroxyquinoline-5-sulfonic acid, 0.5 $\gamma$ of erythrosine, 0.5 $\gamma$ of diiodotyrosine, and 1 $\gamma$ of 4-iodomandelic acid, iodoacetanilide, and p-iodaoniline.

Other halogens do not interfere with this detection of organically bound iodine. When the test is used, it is necessary to take into account only oxidizing compounds which liberate iodine from acidified potassium iodide solutions. Such interfering materials are chloranil, bromanil, organic per compounds, and organic derivatives of arsenic acid. A preliminary test for such oxidants can be made by stirring a drop of the test solution, or a little of the solid, with acidified potassium iodide solution. If iodine is liberated, a new portion of the sample should be taken to dryness with an excess of sulfurous acid and the evaporation residue tested by the procedure just outlined. However, organic derivatives of arsenic acid cannot be rendered harmless in this manner.

### ACKNOWLEDGMENT

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# Detection of Antipyrine and 1-Naphthylamine

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Nitrosoantipyrine condenses in acetic acid solution with 1-naphthylamine hydrochloride to give a violet azo dye. The color reaction, which may also be conducted as a spot test, can be made the basis of a specific test for antipyrine and also for 1-naphthylamine. The procedure described here for the detection of antipyrine probably will make possible a general test for C-nitrosable compounds.

KNORR (1) discovered that antipyrine (2-3-dimethyl-1-phenyl-3-pyrazolin-5-one) is readily nitrosated by nitrous acid:
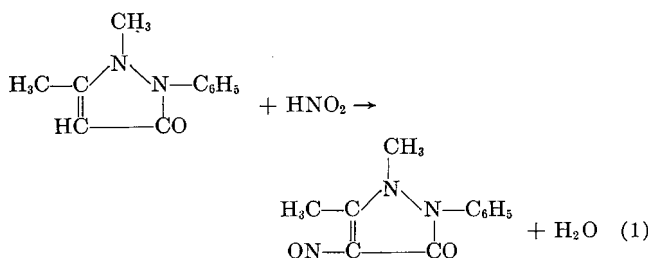


He likewise found that the resulting green, acid-soluble nitrosoantipyrine can serve to detect antipyrine at dilutions down to 1 to 10,000. Attempts to apply this test in spot test analysis gave identification limits of 40 γ of antipyrine, a limit that is not satisfactory for microanalytical objectives. Furthermore, the Knorr test is not reliable in the presence of pyramidone (aminopyrine- 4 - dimethylamino - 2,3 - dimethyl - 1 - phenyl - 3 - pyrazolin - 5 - one), which yields a transient violet color with nitrous acid.

As aromatic nitroso compounds condense with primary amines to form azo compounds (4) and the pyrazolone ring has aromatic character, it is likely that the reaction between nitrosoantipyrine and 1-naphthylamine likewise involves the formation of an azo (pyrazol) dye:
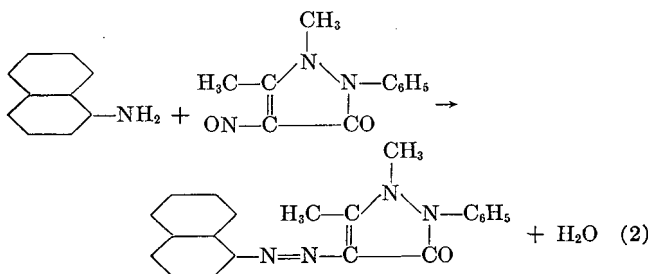


Color Reaction 2 can be used to detect not only antipyrine (after nitrosation) but also 1-naphthylamine with remarkable specificity and sensitivity.

A need for detecting antipyrine and its salts arises particularly when medicinals are being examined because it sometimes is necessary to distinguish it from pyramidon, which has similar analgesic and antipyretic action. The procedure given here serves this purpose well, and it can be conducted with small amounts of solid preparations. The authors found that 0.1 to 0.5 mg. of pyramidon, obtained from various sources, yielded a very faint, violet color. This was probably due to a slight content of anti-

pyrine, which is used as starting material for the preparation of pyramidon.

## DETECTION OF ANTIPYRINE

The first step in the detection of antipyrine is nitrosation in acetic acid solution as per Reaction 1. The excess nitrous acid must be removed or destroyed prior to the condensation (Reaction 2), as otherwise the naphthylamine will be diazotized and coupling with excess amine will occur. Nitrous acid can be completely destroyed by adding sodium azide to the acidic solution.

$$HNO_2 + HN_3 \rightarrow H_2O + N_2O + N_2 \qquad (3)$$
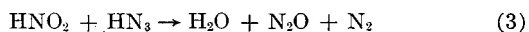
This reaction first recommended by Sommer and Pincus (3) occurs at room temperatures almost instantaneously and there is no effect on the nitrosoantipyrine.

**Procedure.** One drop of the test solution is placed in a micro test tube along with 1 drop of glacial acetic acid, and 1 drop of a 5% solution of sodium nitrite is added. After 5 minutes, with occasional shaking, a pinch (tip of knife blade) of sodium azide is added, and the mixture is allowed to stand until there is no more evolution of gas. Several milligrams of solid 2-naphthylamine hydrochloride are then added and the tube is warmed for a minute or two in a water bath. An intense or faint violet color develops according to the quantity of antipyrine present. When tiny amounts are suspected, it is best to run a comparison blank test. The identification limit is 2.0 γ of antipyrine. Dilution limit is 1 to 25,000.

## DETECTION OF 1-NAPHTHYLAMINE

The procedure will reveal the presence of 1-naphthylamine and its salts such as chloride and sulfate. In the presence of mineral acid, the color reaction is weakened or prevented entirely, depending on the free acid content. However, the addition of solid sodium acetate to a mineral acid solution of 1-naphthylamine restores its capability of condensing with nitrosoantipyrine. 2-Naphthylamine does not react, either as free base or in the form of its salts with acetic or mineral acids. Consequently, 1-naphthylamine can be detected in the presence of any amount of 2-naphthylamine.

Aniline acetate condenses with nitrosoantipyrine in an analogous manner to produce a violet azo dye. On the other hand, aniline salts of mineral acids do not react, so that 1-naphthylamine salts can be readily and reliably detected in mixtures with aniline salts. This was demonstrated with a solution which contained, per drop, 5 γ of 1-naphthylamine hydrochloride along with 1500 γ of aniline hydrochloride.

Naphthylaminesulfonic acids likewise react with nitrosoantipyrine, but with far less sensitivity than 1-naphthylamine and its salts. Brown condensation products result. Trials with 1,3- and 1,4-naphthylaminesulfonic acid gave identification limits of 75 γ of naphthylaminesulfonic acid. H-acid (8-amino-1-naphthol-3,6-disulfonic acid) and 1,8-naphthylenediamine yield brown-red condensation products. Accordingly, the violet color seems to be characteristic of 1-naphthylamine. A shift of the color toward brown occurs when acid or basic groups are introduced into the aromatic ring.

**Reagent Solution.** One gram of antipyrine is dissolved in 20 ml. of 1 to 1 acetic acid and 0.6 gram of sodium nitrite added. After 10 minutes' standing, with occasional shaking, 0.5 gram of sodium azide is introduced and the volume brought to 150 ml. with 1 to 1 acetic acid. The reagent solution keeps for several days.

**Procedure.** One drop of the acetic acid solution to be tested is treated in a micro test tube with 1 drop of reagent solution. The sample may be the free base or the hydrochloride. The mixture is warmed in the water bath for 1 to 2 minutes. Depending on the quantity of 1-naphthylamine present, a more or less intense violet coloration develops. A blank is advisable when only slight amounts of 1-naphthylamine are involved. Identification limit is 0.5 γ of 1-naphthylamine. Dilution limit is 1 to 100,000.

The test for antipyrine described here seems to be a special case of a general method for detecting aromatic compounds which yield true C-nitroso derivatives. This follows from the behavior of aniline, diethyl-, and dimethylaniline, phenol, and also 1- and 2-naphthol. Only the first four of these compounds give a distinct violet or brown color after they have been nitrosated, the excess nitrous acid has been removed, and then they have been heated for some time with 1-naphthylamine. The naphthols yield

no more than a faint color and even this result is probably due to the fact that the nitrosonaphthols are not true C-nitroso compounds but instead are, to a large extent, the isomeric quinoximes.

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# Microreflectivity Analysis of Coal

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A proposed objective method of petrographic analysis of coal based on variations in reflectivity of the coal components has been tested. The analysis is made on polished solid blocks or briquets of granular samples, using a specimen scanning device, a reflected light microscope with high resolution of areas of different reflectivity, a photomultiplier photometer, and a recording microammeter. Comparison of results by this method with those of the Bureau of Mines thin-section technique shows that the former can evaluate, probably more objectively than the latter, the proportions of the opaque components that are relatively resistant to hydrogenation and usually have weak coking properties. Thus this new method should be of value in the selection of coals suitable for hydrogenation to gasoline and coal chemicals, and in the control of blending practices for coal carbonization.

PETROGRAPHIC analysis of coal usually has been made either on thin sections by transmitted light or on polished surfaces by reflected light. The former method has been favored in the United States, particularly by the Bureau of Mines (10), while the latter has been developed chiefly by German coal petrographers (5). Each has its advantages and disadvantages. The thin-section technique is characterized by striking color differences that are of great value in differentiating various coal components; the polished-surface method is favored because of easier specimen preparation and better differentiation of constituents that are opaque in thin section.

The German coal petrographers apparently make little use of quantitative reflectivity measurements in their routine analyses, but rely mainly on qualitative estimations of degrees of brightness, together with structural variations to classify the different coal components. Quantitative determinations of coal reflectivity received great impetus from the startling assertions of Seyler (12, 13), who contended that the reflectivities of the woody constituents of all coals were tightly grouped in a discontinuous series and that variations in reflectivity caused either by diagenetic differences or metamorphic changes always occurred in definite steps. Although studies by the Bureau of Mines (9) failed to substantiate Seyler's claims, quantitative measurements of the reflectivities of coal components should have considerable value in improving and supplementing the present methods of petrographic analysis.

Inadequacy of the thin-section method was emphasized by a Bureau of Mines investigation of the value of petrographic analysis in predicting the probable yields of liquid products in the hydrogenation of coal to synthetic fuels and gasoline (4). This study showed that there was a rough correlation between the yield of insoluble residue resulting from hydrogenation and the amount of relatively inert components, opaque matter, and fusain, in the original coal. However, in different coals, the proportions of opaque matter that could be liquefied were found to vary from 40 to 80%, and 10 to 55% of different fusains liquefied. The varying resistance to liquefaction probably depended on the effective rank of the opaque constituents, but no accurate method of determining this was known. However, the degree of opacity was an important factor in determining resistance to hydrogenation and a method for measuring such variations would have considerable value. Unfortunately, the difficulties involved in controlling the thickness of various areas of extremely thin coal sections discouraged attempts to proceed along this line.

Because the reflectivities of coal components are approximately inversely proportional to their translucences in thin section, the possibility of developing a method of petrographic analysis based solely on quantitative measurements of reflectivity was explored. Seyler has published some results of this type (11), showing the proportions of coal samples having reflectivities equivalent to several of the steps of his series. His analyses were made on both solid blocks of coal and granular samples molded into a briquet with a binder. Seyler measured the reflectivity visually with a photometer attached to a microscope and totaled the amounts of each component with a manually operated integrating stage. This appears to be an extremely laborious and time-consuming procedure. The possibility of utilizing a photoelectric detector, together with a recorder, to measure reflectivity variations in a coal sample on a moving microscope stage seemed promising, and with this objective the present investigation was undertaken.

## EXPERIMENTAL PROCEDURE

**Description of Coal Samples.** Descriptions and analyses of coal samples similar to those used in this investigation are shown in Table I. These analyses do not apply strictly to the specimens studied because in most cases these were selected small portions from different locations in the coal beds. The data are given to show the general nature and rank of the specimens.

**Preparation of Coal Specimens.** Because it is advantageous to be able to make petrographic analyses on small granular samples representative of large lots of broken coal, a technique

was developed for binding such samples into a briquet suitable for these studies. Coal passing a U. S. Standard No. 70 sieve was selected as the most suitable size, so as to avoid inordinately long scanning times to cover representative samples of larger sized coal, and excessive proportions of fine particles in samples crushed to smaller top sizes. The coal should be crushed so as to produce a minimum of fine sizes.

A thermoplastic resin known as Santolite MHP (Monsanto Chemical Co.) was selected as a binder after a number of resins and waxes were tried. Its index of refraction is such that the reflectivity is nearly zero in the immersion oil used on the specimen. To prevent reflections from coal particles lying beneath the surface of the transparent Santolite, 5% by weight of a black dye, nigrosine, is dispersed in the resin. The Santolite is also crushed to pass the No. 70 sieve and is mixed with the coal in the proportion of 2 parts by weight to 3 parts of coal. The mixing is done for 20 minutes in a small bottle attached to a revolving wheel to ensure uniform distribution of coal and binder (3). A cylindrical briquet of the mixture, 1 inch in diameter and $1/2$ inch thick, is then molded on a metallurgical mounting press under a pressure of 500 pounds per square inch and at temperatures somewhat above 62° C., the melting point of the Santolite.

A fresh flat surface is exposed on the briquet by grinding on a fine Carborundum lap and further smoothing on a fine Belgian hone. The surface is then polished on a revolving lap covered with Selvyt cloth, using moistened magnesium oxide as the polishing powder. These briquets polish well, the binder becoming fairly uniformly black and the coal particles reasonably free from scratches. Solid pieces of coal are ground and polished in the same manner.

**Method of Analysis.** Figure 1 shows a schematic diagram of the apparatus used in obtaining reflectivity distribution curves of the briquetted or solid coal specimens. A photograph of the equipment is shown in Figure 2. The polished specimen on the microscope stage is moved at a rate of 0.1 mm. per minute by a flexible cable attached to the fine-pitched stage screw and driven by a small motor and gear set. A 20-mm. traverse across the surface is made in each test. The flexible cable is kept taut by the roller and spring arrangement on the drive mount to prevent strains in the stage that disturb the focus on the surface.

Light from a 6-volt, 5-ampere, tungsten-filament lamp, kept constant by a storage battery whose output is balanced by a regulated charging current, is led through a vertical illuminator and reflected from the specimen surface up through a $1/7$-inch oil immersion objective that forms an image of the surface at the microscope ocular. In this plane is placed a very fine resolving pinhole, which has an effective diameter of 1.85 microns at the specimen. Thus a circular area of this diameter is exposed to the photomultiplier detector fastened above the ocular. The output of the photomultiplier is fed through an amplifier to a Speedomax recorder, which balances and records full-scale deflections in 1 second. As the specimen moves at a rate displacing the exposed area its own diameter in the 1 second necessary to record, a curve is drawn on the recorder chart of the variations in reflectivity of the specimen surface. Figure 3 shows a sample test record, representing about 0.4 mm. of the traverse. At intervals of about 20 minutes during the test, the specimen is observed visually through the side observation tube shown and slight variations from focus are corrected. The zero setting of the photometer amplifier, which drifts slightly, is also adjusted at this time. A test requires about 3.5 hours.

The chart is calibrated by recording the reading on a polished surface of calcium tungstate, whose reflectivity is similar to that of coal. This is done before and after a test, because an appar-
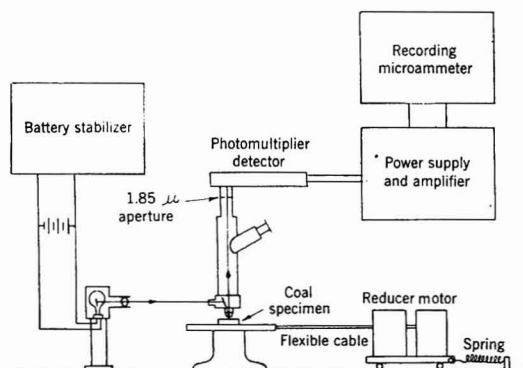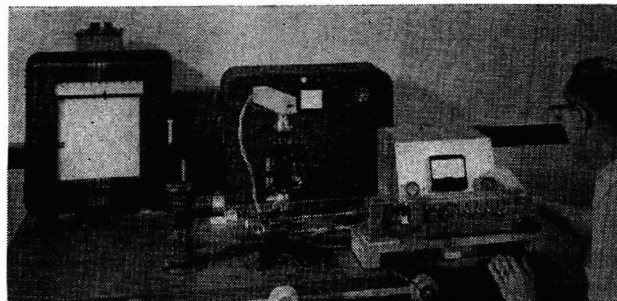


**Figure 2. Microreflectivity apparatus**

ently unavoidable loss of sensitivity occurs owing to fatigue of the photomultiplier tube. A correction is applied to the test record, assuming that loss of response is linear with time. The chart is analyzed by measuring manually with a map measure the lengths of the regular chart graduations that lie below the curve. Because each line represents a fixed value of reflectivity as determined from the calibration, the data can be treated to obtain the percentages of the specimen that have reflectivities greater than these arbitrary values. With briquetted specimens, the results are corrected to represent only the coal in the briquet, using the volume ratios of coal and binder as determined from their weights and densities. A curve showing the distribution of components of different reflectivity in the coal can then be plotted.

Coal reflectivities are measured in cedar oil because the contrast between the components is greatly improved over that obtained with a dry objective (14). Although the total reflectivities are reduced under oil, the ratios of those of the different components are increased, thus aiding in differentiation. The absolute reflectivities are not obtained because monochromatic light is not used. It is preferable to use as much light as possible, so that maximum resolution can be attained on the finest coal particles and on finely divided attrital matter. The values of reflectivity used in the graphs of this paper are based on that of calcium tungstate in sodium light as determined in a previous study (9). The resolution of 1.85 microns is attained by utilizing the photomultiplier photometer at maximum sensitivity. The output of the photomultiplier tube, after nine stages of amplification within the tube, is of the order of $10^{-9}$ ampere.
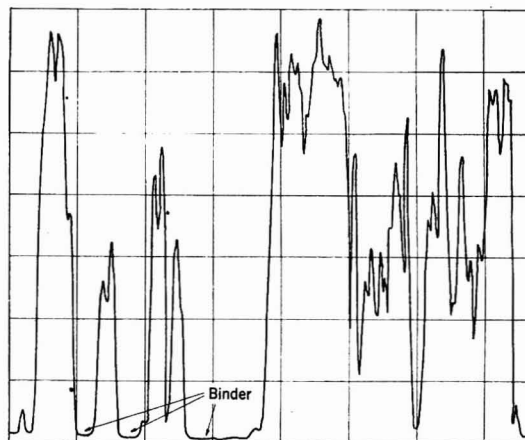


**Figure 3. Chart record of reflectivity variations in briquet of granular coal**

Many of the measurements involved in this procedure are near the limits of performance of available instruments. The size of the resolving aperture was chosen such that the reading of the photometer meter on the most sensitive range was full scale for the calcium tungstate reflectivity standard. Thus the resolution at the specimen, 1.85 microns, is limited by the sensitivity of the photomultiplier. By using a more intense light source or a more efficient condenser, this resolution might be improved, but in any case the limit of resolution of this optical microscopic sys-



**Figure 1. Schematic diagram of equipment for microreflectivity analysis of coal**
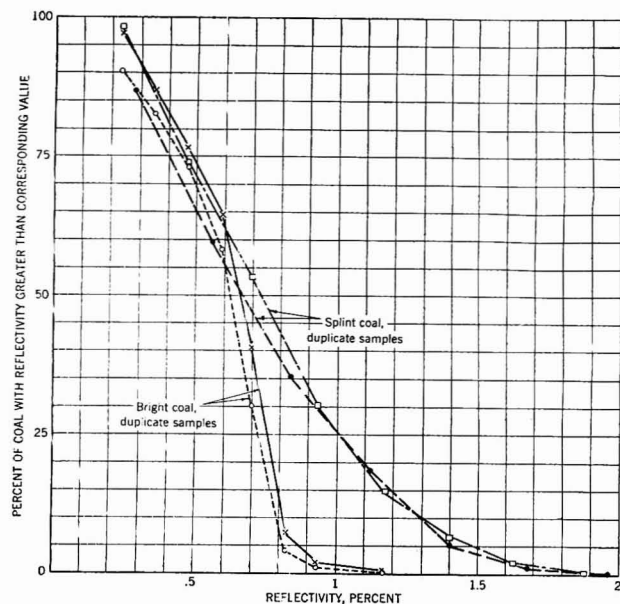
**Figure 4. Reflectivity distribution curves**

Duplicate briquetted samples of granular coal from bright and splint layers of Alma high-volatile A coal
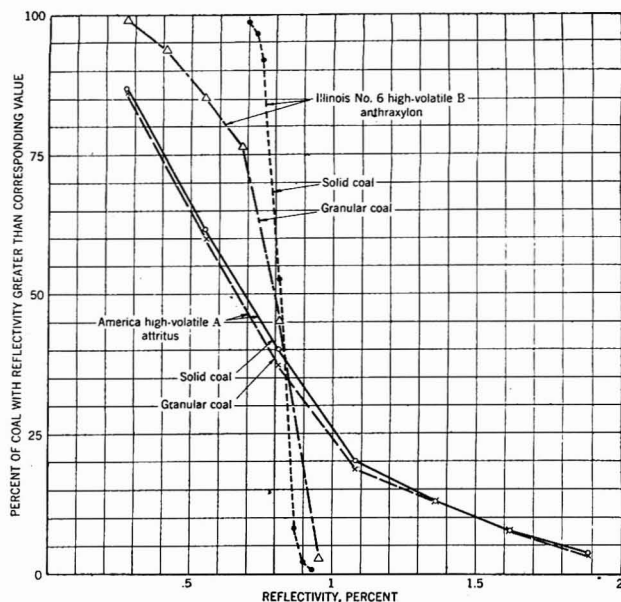


**Figure 5. Reflectivity distribution curves**

Solid blocks and granules from two coals of different type
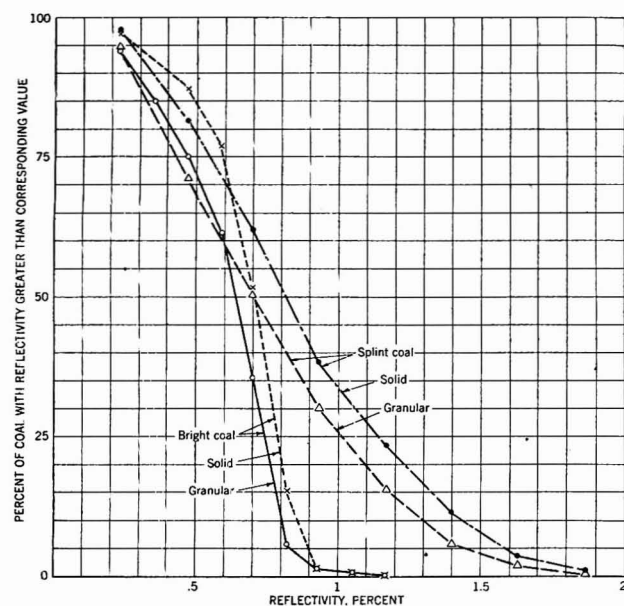


**Figure 6. Reflectivity distribution curves**

Solid blocks and granules from bright and splint layers of Alma high-volatile A coal
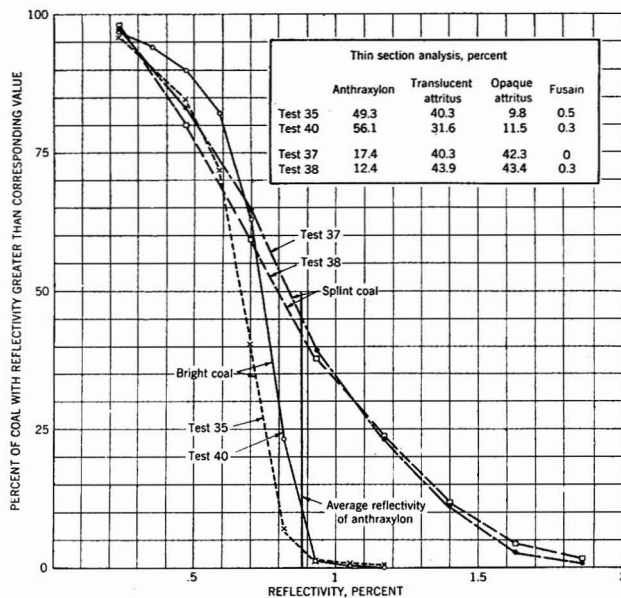


**Figure 7. Reflectivity distribution curves**

Adjacent solid blocks from bright and splint layers of Alma high-volatile A coal

tem, about 0.5 micron, prevents improvement beyond that point. Because mechanical scanning of a somewhat uneven specimen surface is involved, resulting in uncertainty of focus, the maximum resolution of the microscope under these conditions is probably not much better than 1 micron. Improved light sources, microscope objectives with higher numerical aperture, more sensitive photometers, or more even specimens can under no circumstances improve the resolution of the measurements by more than a factor of 4 and probably by not more than 2.

The speed of scanning is given by the scanning area diameter, 1.85 microns, divided by the response time of the recorder, 1 second, or 1.85 microns per second. This scanning speed is therefore limited by the recorder response time, which for the fastest potentiometer-type recorders is now about 1 second. Direct-recording galvanometer types are faster but correspondingly less accurate. A major improvement could be gained by substituting electronic integrating circuits for the relatively

slowly responding potentiometer recorder. By the use of counters or timers that would start and stop at certain values of reflectivity, the test time could be greatly reduced and mechanical analysis of a chart eliminated.

The length of scan is taken as about 100 times the diameter of the largest particle in a granular specimen. This is done so that the scan will be subject to statistical fluctuations of not more than 1%. The number of scans necessary for any desired accuracy depends on the representativeness of the sample, the homogeneity of the briquet, and the quality of the polish.

The error resulting when the particles being scanned are not much larger than the scanning area causes a false contour of the recorded reflectivity curves, which may be described in a simplified way. If a scanning area of diameter $A$ microns is used to scan a particle $D$ microns in diameter, the particle will appear to be larger in diameter by $A$ microns and it will register its true reflectivity for a distance of $A$ microns less than the true diameter of

## Table I. Description and Analyses of Coal Samples

| Coal Bed | Mine | County | State | Rank | Proximate Analysis, % | | | | Ultimate Analysis, % | | | | | Calorific Value, B.t.u. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Moisture | Volatile matter | Fixed Carbon | Ash | Hydrogen | Carbon | Nitrogen | Oxygen | Sulfur | |
| ... | ... | ... | Alberta, Can. | Lignite | 13.1 | 38.0 | 42.5 | 6.4 | 5.1 | 56.3 | 1.2 | 30.5 | 0.5 | ... |
| A | Wise Hill No. 2 | Moffat | Colo. | Subbituminous A | 13.4 | 30.8 | 46.9 | 8.9 | 5.5 | 60.2 | 1.2 | 23.5 | 0.7 | 10,470 |
| No. 6 | Old Ben No. 15 | Franklin | Ill. | High-volatile bituminous B | 10.3 | 33.6 | 48.6 | 7.5 | 5.4 | 67.3 | 1.5 | 17.0 | 1.3 | 11,900 |
| No. 5 | Thermond | Gallatin | Ill. | High-volatile bituminous A | 1.6 | 37.3 | 51.8 | 9.3 | 5.2 | 73.4 | 1.5 | 7.3 | 3.3 | 13,260 |
| America | America No. 3 | Walker | Ala. | High-volatile bituminous A | 2.6 | 31.0 | 49.8 | 16.6 | 4.7 | 67.1 | 1.5 | 8.4 | 1.7 | 12,070 |
| Alma | Red Jacket No. 6 | Mingo | W. Va. | High-volatile bituminous A | | | | | | | | | | |
| Layer 2 (splint) | | | | | 1.1 | 39.2 | 57.0 | 2.7 | 5.4 | 83.1 | 1.3 | 5.9 | 0.5 | 14,930 |
| Layer 3 (mixed bright and splint) | | | | | 1.2 | 36.4 | 59.2 | 3.2 | 5.4 | 82.1 | 1.5 | 5.9 | 0.7 | 14,690 |
| ... | ... | ... | Alberta, Can. | Low-volatile bituminous | 0.6 | 16.5 | 78.0 | 4.9 | 4.4 | 84.6 | 1.3 | 4.3 | 0.5 | ... |
| Hillman | Henry | Luzerne | Pa. | Anthracite | 4.0 | 5.7 | 85.8 | 4.5 | 3.0 | 85.9 | 0.9 | 5.1 | 0.6 | 13,880 |

the particle. The total error on the complete record will be larger with greater proportions of fine particles.

### RESULTS

**Duplicability.** The reproducibility of results from this type of test was first determined for two adjacent 20-mm. traverses on the same briquet. Reflectivity distribution curves for two traverses on a briquet of selected anthraxylon (vitrain) from a high-volatile B coal from the No. 6 bed of Illinois showed an average difference of 1%. A difference of 3% was found for two traverses on a briquet of splint coal from the America bed of Alabama. This appears to be very satisfactory reproducibility on the same specimen, and the deviation is understandably much less for the uniform anthraxylon than for the heterogeneous splint coal. The duplicability for traverses on two different briquets is shown in Figure 4 for crushed samples from bright and splint layers of Alma-bed high-volatile A coal from West Virginia. Here are introduced the additional possible differences of sampling and polishing in the two briquets. The agreement is still good, the average difference for both the bright and splint-coal briquets being about 6%. This order of reproducibility for single traverses on two different briquets is reasonable. Of course, a statistical study should be made of results for a number of traverses on briquets containing coals of different ranks and types to determine the number of traverses required for a given allowable deviation. There is little or nothing in the literature describing the application of lineal analysis to specimens of this type.

**Comparison of Results on Briquets and Solid Blocks.** Comparative results on solid pieces of coal and briquets of granular (minus No. 70-sieve) coal from adjacent locations in the bed show the effects of possible errors inherent in the analysis of granular coal. Figure 5 shows such reflectivity-distribution curves for Illinois No. 6-bed high-volatile B anthraxylon, the blocks and granules of which are fairly homogeneous, and for an America high-volatile A attrital sample, which consists of very finely disseminated coal material. The curves for the solid and granular anthraxylon are reasonably similar through most of the range but with marked deviation of the upper portion of the granular coal curve toward lower reflectivity values. This is probably caused by the previously described edge effects between dark binder and brighter coal. The difference between the reflectivity distributions of the solid and granular attrital coal is much less than for the anthraxylon, possibly because insufficient resolution of very fine detail in the coal itself, both in solid and granules, masks the effect of discontinuity in the granular coal. Figure 6 shows a similar comparison for bright and splint layers from the same bed of Alma high-volatile A coal. Here the differences between solid and granular samples appear to be greater, even though these curves represent the averages of traverses on two different blocks or briquets, while those in Figure 5 are for single tests. In both these samples of Figure 6, the structural details are larger than in the splint sample of

Figure 5, so that resolution is better for both blocks and granules, and the difference must be caused mostly by particle resolution rather than structural detail resolution. It is also possible that the block and granule samples are not quite the same, since they come from different, though adjacent, locations. The curves for the anthraxylon of Figure 5 may be closer because it does not all have the same reflectivity, and the selected solid piece may have a lower than average reflectivity, as is indicated by the granular curve showing high reflectivity near the base of the graph.

The average order of agreement between solid and granular coal is probably better represented by Figure 6. The differences are appreciable, but the changes in the distributions are parallel. Therefore, although the results on granular coal are lower than the true values, the errors on most coals should be reasonably comparable and the great advantage of analyzing small granular samples over large columns of solid coal may outweigh the errors involved.

Figure 7 shows a comparison of reflectivity-distribution curves for traverses on two blocks of solid coal selected from adjacent locations in the same layer. The distributions for the traverses on the splint coal agree closely, probably because the average distribution of the finely divided attritus does not vary much within the same layer. The bright-coal distributions deviate more, probably because there is more chance of variation, since some relatively large anthraxylon bands may not be continuous over appreciable distances. Actually the two bright-coal traverses were made about $1/2$ inch apart on the same block, but there was an evident anthraxylon band about $1/8$ inch wide that was crossed by only one of the traverses. In general, two traverses on adjacent solid-coal pieces would be expected to vary more than those on two briquets of the same sample of granular coal.

The possibility of preparing briquets of granular coal by means of high pressures without the use of binder was investigated. Briquets of minus No. 200-sieve coals of different types were prepared at 200,000 pounds per square inch. A good polish on these briquets was difficult to obtain, and the high pressure apparently caused excessive alteration of structural details. A trial test on one of these briquets gave a reflectivity-distribution curve that had a lower average reflectivity than that of a minus No. 200-sieve briquet with binder. It was tentatively concluded that such high-pressure briquets would be unsuitable for these reflectivity studies.

**Effect of Rank of Coal.** Previous investigators have shown that the reflectivity of anthraxylon or vitrain varies more or less regularly with rank (6). Figure 8 shows reflectivity distribution curves for coals ranging in rank from lignite to anthracite. The upper parts of the curves for anthracite and low-volatile bituminous coal are apparently in error because of insufficient resolution on fine particles and the relatively large difference between the reflectivities of the coal and binder. Coals of high-volatile A rank and lower appear to be less seriously affected by this error. The coal samples represented are not strictly comparable by type,

for some are bright coals and others are splint or semisplint coals. The average reflectivities increase with rank, but there is considerable overlapping. The high-volatile A samples contain some components of higher reflectivity than much of the low-volatile coal, and the subbituminous sample has components of greater reflectivity than some in the high-volatile coals.
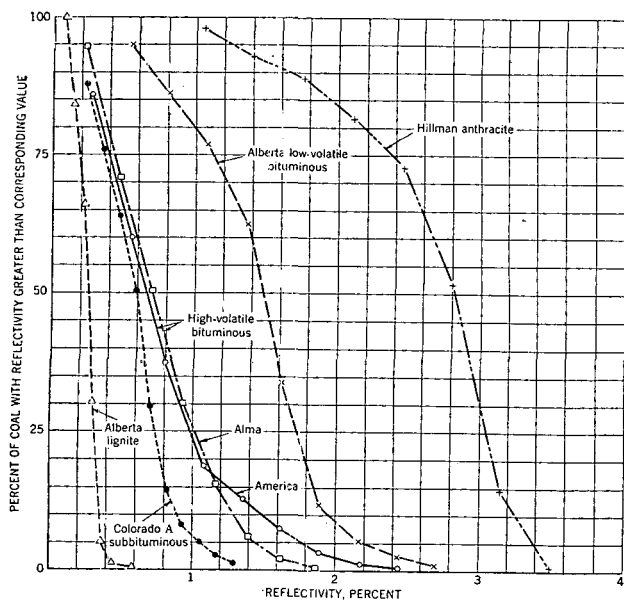


**Figure 8. Distribution of components of varying reflectivity in coals of different rank**

A complicating factor in studies of reflectivity of coal arises with the appearance of anisotropy in higher rank coals. Cannon and George (1) found evidence of anisotropy in a coal of 25% volatile matter, but it did not seem appreciable in coals below semianthracitic rank. Dahme and Mackowsky (2) classified coals with 10 to 18% volatile matter as weakly anisotropic and those below 10% as strongly anisotropic. Van Krevelen (7) stated that coal is anisotropic at a carbon content above 87%, but his data do not show appreciable anisotropy below about 91% carbon. Apparently anisotropy becomes marked only in the anthracitic coals, but may be slightly evident in coals of low- and medium-volatile rank. This phenomenon is probably of little importance for most granular samples that would be treated by the type of measurement described here, but the possible variations of reflectivity in different directions with the bedding planes should be considered in most discussions of coal reflectivity.

**Effect of Type of Coal.** The efficacy of this method of analysis in distinguishing between different types of coal is of great interest. Figures 4 to 7 offer evidence on this point. Figures 4, 6, and 7 show the differences between reflectivity distribution curves for samples of bright and splint coal from the same bed. The splint coal obviously contains a large proportion of components with greater reflectivity than those of the bright coal. This agrees with the conception that opaque constituents that are more abundant in the splints have higher reflectivities than the more translucent components of the bright coals. To compare the results by this method more accurately with those obtained in the standard Bureau of Mines thin-section analysis, the solid blocks on which reflectivity data were obtained were subsequently used to prepare thin sections, and transects were made along approximately the same 20-mm. lengths as were traversed in the reflected light studies. The data obtained thereby are tabulated in Figure 7. The greatest differences between the bright and splint samples lie in their contents of opaque attritus. More

than 40% of the splint coals but only 10% of the bright pieces consist of opaque constituents. A useful criterion for assessing the meaning of the reflectivity-distribution curves as related to translucence might lie in the average reflectivity of the main anthraxylon component of the coal. For the Alma high-volatile A coal this was determined on relatively wide uniform bands of anthraxylon and found to be approximately 0.88%. Inspection shows that about 44% of the components in the solid splint-coal samples have reflectivities higher than that of the major anthraxylon component, while about 4 to 11% of the bright samples have higher reflectivities. It is undoubtedly somewhat fortuitous that these figures agree so well with the opaque-matter contents determined by thin-section analysis, but this appears to be a useful method of interpretation.

Figure 5 also shows the differences between two coal samples of different type. The Illinois anthraxylon has nearly uniform reflectivity throughout, while the sample of Alabama attrital coal selected from a splint layer has components of a wide range of reflectivity. Spores or exinite in this sample have low reflectivity and opaque matter has high reflectivity.

A source of error that has not been mentioned is the presence of mineral matter in the coal. Its reflectivity is usually high, but it would be difficult to recognize and subtract its contribution to the reflectivity record. It probably would be desirable to remove a good deal of the mineral matter from the granular sample by gravity separation methods. However, if the mineral content is not excessive, not over 10% by weight, its contribution to the reflectivity analysis based on volume per cent would be usually less than 4% and differences between most coals would be much less than this.

**Possible Applications of Results.** The most obvious application of results obtained by this method of analysis appears to lie in prediction of the amenability of coals to hydrogenation into liquid fuels. As previously discussed, the constituents most resistant to liquefaction are those that are opaque in thin section and have relatively high reflectivities. The quantities obtainable from the reflectivity distribution curves that might be tried for correlation with yields of insoluble residues in hydrogenation tests are the proportions of the coals having reflectivities greater than their main anthraxylon component, the proportions with reflectivities greater than a fixed value, and the relative distribution of these higher reflectivity components. To illustrate the latter, the curves for the two high-volatile bituminous coals in Figure 8 are of interest. The Alma and America coals have about the same proportions of constituents with reflectivities greater than that of the anthraxylon, which is about 0.88% in both coals. However, the America coal has greater amounts of higher reflective components—for instance, 12% of the America coal has reflectivity above 1.40%, while only 6% of the Alma coal has this reflectivity, and this contrast continues to higher reflectivity values. Characteristics such as these may be found to have valuable application to prediction of hydrogenation yields.

The potential usefulness of the Bureau of Mines thin-section technique in assaying coals proposed for hydrogenation has been discussed. This new reflectivity method, besides being more objective, apparently can characterize more critically the opaque components that are difficult to liquefy. To further explore the value of this method, a program of tests should be conducted to determine the possible correlations with the yields obtained in small scale hydrogenation tests. If a good and useful relationship can be demonstrated, this analytical method would offer a simple, inexpensive means of determining the relative merits of different coals that are economically available for the production of gasoline and coal chemicals by hydrogenation.

This more objective method of actually measuring the reflectivity variations in a coal sample should also be of value in supplementing the reflected-light methods of petrographic analysis that are favored by European investigators and are currently attracting attention in this country. German petrographers, in

particular, are making extensive use of these techniques in controlling the blending of coals for the manufacture of metallurgical coke. Van Krevelen (8) in The Netherlands has recently proposed a simplified form of presentation of the results of petrographic coal analysis that he believes has considerable practical value to those concerned with problems of preparing coals and blends for carbonization. Van Krevelen would report an analysis with figures such as these: 5-721, where the number 5 represents the reflectivity or rank of the vitrinite (anthraxylon) in the coal on a scale of 0 to 9 similar to Seyler's steps, and the figures 721 represent 70% vitrinite, 20% exinite (spores), and 10% inertinite (opaque matter) as the petrographic composition of the coal. Because the exinite is of lowest reflectivity, the vitrinite intermediate, and the inertinite of highest reflectivity, the reflectivity distribution curves obtained by the method presented here should be amenable to an interpretation that will furnish data similar to that proposed by Van Krevelen.

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# Field Determination of Microgram Quantities of Niobium in Rocks

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A rapid, simple, and moderately accurate method was needed for the determination of traces of niobium in rocks. The method developed is based on the reaction of niobium(V) with thiocyanate ion in a 4M hydrochloric acid and 0.5M tartaric acid medium, after which the complex is extracted with ethyl ether. The proposed procedure is applicable to rocks containing from 50 to 2000 p.p.m. of niobium, and, with modifications, can be used on rocks containing larger amounts. Five determinations on two rocks containing 100 p.p.m. or less of niobium agree within 5 p.p.m. of the mean, and the confidence limits at the 95% level are, respectively, ±6 and ±4 p.p.m. The addition of acetone to the ether extract of the niobium thiocyanate inhibits the polymerization of the thiocyanate ion and stabilizes the solution for at least 20 hours. The proposed procedure permits the determination of 20 γ of niobium in the presence of 1000 γ of iron, titanium, or uranium; 500 γ of vanadium; or 100 γ of tungsten or molybdenum or both.

AS PART of its contribution to a commodity study of certain strategic materials, the U. S. Geological Survey is developing geological information concerning the element niobium. This program required a field screening of potential niobium-bearing materials, in order to eliminate shipment of barren samples to the laboratory and to permit the field geologists to make appropriate adjustments in their exploration program with minimum delay. A rapid semiquantitative method for determining traces of niobium in rocks was needed for this screening operation. Until recently the determination of traces of niobium by chemical methods was unsatisfactory because the available reactions were not sufficiently sensitive, and the niobium had to be separated from numerous interfering elements such as tantalum, vanadium, uranium, molybdenum, and tungsten. Often these separations were incomplete. Chromatographic procedures (8), which offer a slightly different approach, are now used to separate niobium

from some of the interferences and to concentrate it on a suitable absorbent. The niobium can then be estimated by a colorimetric method based on the reaction of niobium either with hydrogen peroxide in strong sulfuric acid (9, 13) or with pyrogallol in alkaline medium (7).

More than 50 years ago Pennington (12) observed qualitatively that niobium formed a yellow color with thiocyanate in an acid medium. Since then several workers (1, 3, 10) have made significant contributions to better methods of determining niobium with thiocyanate. Freund and Levitt (3) studied the effects of different concentrations of acid, stannous chloride, and thiocyanate on the reaction in an acidic-acetone-water mixture. Lauw-Zecha, Lord, and Hume (10) made a similar study using ethyl ether to extract the niobium thiocyanate from the aqueous solution. Marzys (11) applied the reaction to the determination of small amounts of niobium in low-grade ores and minerals and studied the relative merits of the water-acetone mixture and ether as solvents of the niobium complex. He made involved separations of copper, mercury, platinum, and molybdenum, and concluded that classical methods must be used to determine niobium in the presence of vanadium

The method here presented contains no involved separations and is not subject to interference from vanadium, titanium, or tantalum.

### EXPERIMENTAL

**Stability of Niobium Thiocyanate in Ethyl Ether–Acetone.** The apparent instability of the yellow-colored niobium thiocyanate in ethyl ether is a serious drawback to methods that use the ether extraction to increase the sensitivity and make the reaction more specific. The term "instability" is here used cautiously, because the evidence of such is indirect and no one has demonstrated that the niobium complex changes in any manner.

Freund and Levitt (3) observed that during a given time the total absorbance of an acid solution of niobium thiocyanate remained constant, but the absorbance of the blank increased. Lauw-Zecha, Lord, and Hume (10) observed that the intensity of

the yellow color of an ether solution of niobium thiocyanate changed during the first 0.5 hour and then became essentially constant for 2 hours. The present authors used fresh solutions of the required reagents to produce a yellow-colored ether solution containing 1 p.p.m. of niobium as the thiocyanate complex; during a 20-hour period the absorbance of this solution decreased from 0.42 to 0.39. Another solution containing 2 p.p.m. behaved similarly. However, when reagent solutions more than 1-day old were used, the absorbance of an ether solution of niobium thiocyanate increased from 0.56 to 0.72 during a 20-hour period. All these measurements were made on a Beckman DU spectrophotometer with pure ether in the null cell; hence the values are for the total absorbance of both the thiocyanate complex and the blank.

Presumably the increase in absorbance of the ether solution is associated with the increase in absorbance of the blank. Freund and Levitt (3) attributed the latter to the polymerization of the thiocyanate ion for high temperature and strong acid accelerated the polymerization and the polymer absorbed strongly in the near ultraviolet range.

Evidence for the polymerization of the thiocyanate ion as an interfering side reaction has accumulated. Lauw-Zecha, Lord, and Hume (10) observed that if the aqueous solution were allowed to stand for a long time after the extraction of the niobium thiocyanate, an unextractable yellow color formed in the aqueous solution. In determining uranium by its reaction with thiocyanate, Crouthamel and Johnson (2) observed that stannous chloride in the presence of uranium and ammonium thiocyanate in aqueous solution generated an interference peak at about 375 mμ which became serious as the thiocyanate solution aged.

Some way of inhibiting or compensating for the polymerization of the thiocyanate ion is needed. The prompt extraction of the niobium thiocyanate as suggested by Lauw-Zecha, Lord, and Hume (10) did not solve the problem, possibly because of the appreciable solubility of alkali thiocyanates in ether. Freund and Levitt (3) developed the yellow-colored niobium complex in an aqueous acetone medium because of an implied stabilizing effect of acetone on the niobium thiocyanate, but their procedure did not compensate for the polymerization of the thiocyanate ion as was shown by the increase in absorbance with time.

Acetone, used successfully to stabilize solutions of other metal thiocyanates—for example, molybdenum (4) and uranium (2)—did not seem to stabilize an aqueous solution of niobium thiocyanate, but did stabilize an ether solution of niobium thiocyanate. Accordingly the authors modified the extraction method of Lauw-Zecha, Lord, and Hume (10).

**Table I. Stability of Niobium Thiocyanate in Ether-Acetone Solution**

| Time, Min. | Absorbance of Ether Acetone Solutions Niobium, P.P.M. | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.2 | 0.5 | 1.0 | 2.0 | 3.0 |
| 15 | 0.02 | 0.13 | 0.25 | 0.42 | 0.87 | 1.15 |
| 45 | | 0.11 | 0.23 | 0.40 | 0.83 | 1.10 |
| 60 | 0.02 | 0.11 | 0.23 | 0.40 | 0.85 | 1.12 |
| 90 | 0.02 | 0.11 | 0.24 | 0.41 | 0.84 | 1.08 |
| 120 | 0.02 | 0.12 | 0.25 | 0.42 | 0.86 | 1.14 |
| 150 | 0.02 | 0.11 | 0.24 | 0.42 | 0.87 | 1.13 |
| 180 | 0.02 | 0.12 | 0.25 | 0.43 | 0.87 | 1.14 |
| 1200 (overnight) | 0.04 | 0.14 | 0.28 | 0.45 | 0.89 | 1.21 |

A single extraction of the niobium thiocyanate was made with ethyl ether previously shaken with stannous chloride in 2M hydrochloric acid. After extraction the ether phase was separated, and an equal volume of acetone was added to the ether. Absorbance data on ether acetone solutions of five different concentrations of niobium are given in Table I.

Within limits of experimental error, the ether-acetone solutions of niobium thiocyanate are stable for at least 20 hours. The solutions of niobium thiocyanate in ether-acetone and in ether

alone are similar in wave length of maximum absorption and adherence to Beer's law.

**Interference of Vanadium.** All previous methods (1, 3, 10) call for the addition of stannous chloride to the aqueous solution before addition of thiocyanate; and in the presence of tartaric acid which is needed to complex tantalum, stannous chloride reduces vanadium to vanadium(III), which reacts with thio-

**Table II. Effect of Vanadium on Determination of Niobium with Thiocyanate**

| Present, γ | | Niobium Found, γ Stannous Chloride Added | |
|---|---|---|---|
| Niobium | Vanadium | Before ether extraction | After ether extraction |
| 0 | 20 | 1 | 0 |
| 20 | 20 | 18 | 18 |
| 0 | 100 | 6 | 0 |
| 20 | 100 | 24 | 19 |
| 0 | 500 | 25 | 1 |
| 20 | 500 | 30+ | 19 |
| 0 | 1000 | 30+ | 3 |
| 20 | 1000 | 30+ | 18 |

cyanate to give a yellow compound soluble in ether. Neither vanadium(V) nor (IV) reacts in such a manner. The absorption peak of the yellow compound formed from vanadium(III) is near 400 mμ, causing it to interfere seriously with the measurement of niobium. Alimarin and Podvalnaja (1) observed, however, that the addition of a reducing agent to the aqueous phase was unnecessary to effect a reaction between the niobium and the thiocyanate. The omission permits the vanadium to remain in the higher valence form, which causes no interference.

The data in Table II show the extent to which the vanadium interference has been eliminated in the proposed method. Although the ethyl ether used to extract the soluble metal thiocyanates is shaken with a small amount of stannous chloride in 2M hydrochloric acid to remove traces of peroxide, these data show that the small amount of stannous chloride in the organic solvent is insufficient to cause appreciable reduction of the vanadium in the aqueous phase.

**Table III. Effect of Iron on Determination of Niobium with Thiocyanate**

| Niobium Present, γ | Iron Present, γ | Niobium Found, γ |
|---|---|---|
| 0 | 20 | 1 |
| 20 | 20 | 16 |
| 0 | 100 | 1 |
| 20 | 100 | 18 |
| 0 | 500 | 1 |
| 20 | 500 | 20 |
| 0 | 1000 | 1 |
| 20 | 1000 | 20 |

**Interference of Iron.** After a bisulfate fusion and in the absence of reducing agents, the iron present in a sample solution is predominantly in the higher valence form. When the aqueous solution is shaken with the ethyl ether to extract the niobium thiocyanate, the red-colored iron thiocyanate is also extracted and is removed conveniently by shaking the ether phase successively with small portions of stannous chloride in 2M hydrochloric acid. Stannous chloride reduces the iron(III) to iron(II) and experiments show that practically all of the iron returns to the aqueous phase. Theoretically, the amount of iron that could be removed by successive treatments with the stannous chloride reagent is limited by the various distribution coefficients and solubilities; however, three treatments are sufficient to permit the determination of 20 γ of niobium in the presence of 1000 γ of iron as shown by the data in Table III.

**Table IV. Effect of Other Elements on Determination of Niobium with Thiocyanate**

| Element | Amount of Element Added, γ | Niobium, γ Added | Niobium, γ Found |
|---------|---------|-------|-------|
| U | 20 | 0 | 0.5 |
|   | 20 | 20 | 18 |
|   | 100 | 0 | 1 |
|   | 100 | 20 | 18 |
|   | 500 | 0 | 1 |
|   | 500 | 20 | 19 |
|   | 1000 | 0 | 1 |
|   | 1000 | 20 | 19 |
| Ti | 20 | 0 | 0.5 |
|    | 20 | 20 | 19 |
|    | 100 | 0 | 0.5 |
|    | 100 | 20 | 19 |
|    | 500 | 0 | 0 |
|    | 500 | 20 | 20 |
|    | 1000 | 0 | 0 |
|    | 1000 | 20 | 18 |
| Ta | 2 | 0 | 0 |
|    | 2 | 20 | 22 |
|    | 20 | 0 | 0 |
|    | 20 | 20 | 22 |
|    | 200 | 0 | 1 |
|    | 200 | 20 | 21 |
| Mo | 20 | 0 | 0.5 |
|    | 20 | 20 | 18 |
|    | 100 | 0 | 3 |
|    | 100 | 20 | 22 |
|    | 500 | 0 | 23 |
|    | 500 | 20 | 38 |
| W | 20 | 0 | 2 |
|   | 20 | 20 | 16 |
|   | 100 | 0 | 7 |
|   | 100 | 20 | 22 |
|   | 500 | 0 | 10 |
|   | 500 | 20 | 26 |
| Bi | 20 | 0 | 0.5 |
|    | 20 | 20 | 22 |
|    | 100 | 0 | 0 |
|    | 100 | 20 | 23 |
|    | 500 | 0 | 1 |
|    | 500 | 20 | 24 |

**Other Interferences.** In order to know the extent of these interferences, varying amounts of the interfering elements (uranium, titanium, tantalum, molybdenum, tungsten, and bismuth) were taken through the entire procedure including the initial fusion in the absence and presence of 20 γ of niobium. The results are shown in Table IV.

**Table V. Repeatability of Niobium Determinations**

| Sample No. | Niobium, P.P.M. 1 | 2 | 3 | 4 | 5 | Mean | S | 95% level |
|------|------|------|------|------|------|------|------|------|
| 1 | 75 | 75 | 67 | 70 | 70 | 71 | 3.5 | ± 4.4 |
| 2 | 80 | 105 | 100 | 75 | 88 | 90 | 13 | ± 16.2 |
| 3 | 90 | 90 | 100 | 95 | 100 | 95 | 5 | ± 6.2 |
| 4 | 350 | 320 | 380 | 310 | 300 | 332 | 32.6 | ± 40.5 |
| 5 | 500 | 550 | 515 | 450 | 600 | 523 | 56 | ± 69.6 |
| 6 | 700 | 850 | 850 | 1000 | 850 | 850 | 106 | ±131.8 |
| 7 | 1450 | 1675 | 1725 | 1675 | 1500 | 1605 | 121 | ±150.4 |
| 8 | 1800 | 1700 | 1900 | 1500 | 1300 | 1640 | 241 | ±299.6 |
| 9 | 8250 | 8250 | 9250 | 8500 | 8500 | 8550 | 411 | ±511 |

Molybdenum and tungsten are serious interferences in the proposed procedure. They can be detected by adding a few drops of an aqueous solution of rhodamine B to a dilute hydrochloric acid solution of the rock sample. The red color of the rhodamine B is changed to a violet color by either molybdenum or tungsten or both. The reaction of molybdenum with thiocyanate normally takes place in a more dilute acid medium than the proposed niobium method provides (15). Conversely, the reaction of tungsten with thiocyanate requires a greater acidity than the proposed method provides, and also some tungsten may be complexed by the tartaric acid.

The data in Table IV show that as little as 20 γ of molybdenum in the presence of an equal amount of niobium depresses slightly the color of the niobium thiocyanate. The effect of 100 γ of molybdenum on the estimation of 20 γ of niobium is not serious; however, the effect of 500 γ of molybdenum under similar circumstances is very serious because it enhances the color of the organic solvent sufficiently to cause high results. In a general way tungsten behaves similarly to molybdenum, although with small amounts of tungsten the depression is more marked, and with large amounts the color is less pronounced.

The data in Table IV show that uranium(VI) is not a serious interference in the proposed method. The sexivalent uranium reacts with thiocyanate to form a complex that fortunately is not readily soluble in ethyl ether (14).

These data also show little interference from titanium but, a peculiar behavior of a standard solution of titanium(IV) was observed. In the presence of chlorides, titanium(IV) is not extractable with ethyl ether (5), and the reduction potential of stannous chloride in 2M hydrochloric acid is not great enough to reduce titanium(IV); nevertheless, when the ether solution of the various metal thiocyanates was shaken with a 2M hydrochloric acid solution of stannous chloride, the aqueous phase was colored violet, indicating titanium(III).

On the basis of the data in Table IV, tantalum and bismuth cannot be considered as serious interferences.

## REAGENTS AND APPARATUS

Acetone, A.C.S. grade.

Ethyl Ether. Unless specially packaged, all dry ethers on standing tend to form explosive peroxides. Peroxides of ethyl ether are dangerous and interfere with the niobium method. To test for peroxides, shake 5 ml. of ether with 5 ml. of an acidified aqueous solution of potassium iodide. If the aqueous solution shows more than a faint yellow color, due to free iodine produced by the reaction of peroxides with iodide, the ether contains appreciable quantities of peroxides and should not be used. Shake ether with one tenth its volume of stannous chloride reagent on the day it is to be used.

Ammonium Thiocyanate. Prepare daily by dissolving 20 grams of the salt in 100 ml. of water.

Hydrochloric Acid–Tartaric Acid Reagent. Dissolve 15 grams tartaric acid in 100 ml. of 9M hydrochloric acid.

Sodium Bisulfate, fused, reagent quality. Grind to a powder to facilitate mixing with samples.

Stannous Chloride. Dissolve 10 grams of the dihydrate in 100 ml. of 2M hydrochloric acid. Filter to remove insoluble stannic salts. A small amount of tin helps to stabilize the solution, but fresh solutions should be prepared every other day.

Standard Niobium Solution, 0.02%. Solution A. Prepare by fusing 0.0286 gram of niobium pentoxide with 1.5 grams of fused sodium bisulfate in a porcelain or preferably a Vitreosil crucible. Dissolve the fused mass in 1M tartaric acid and dilute to 100 ml. with 1M tartaric acid. This solution contains 200 γ of niobium per ml.

Standard Niobium Solution, 0.002%. Solution B. Prepare by diluting 10 ml. of solution A to 100 ml. with 1M tartaric acid and mixing thoroughly. This solution contains 20 γ of niobium per ml.

Tartaric Acid, 1M. Dissolve 15 grams of tartaric acid in water and make up to 100 ml.

Balance, sensitivity of 2 mg. or better.

Borosilicate culture tubes, 18 × 150 mm.

One borosilicate volumetric flask with stopper, 100-ml. capacity.

Twelve borosilicate volumetric flasks, 10-ml. capacity.

One camel's hair brush, 18-mm. length of brush part.

One digestion and fusion rack to support 8 to 10 tubes over the gasoline stove.

Glass filtering fiber, fineness AAA.

Mullite mortar and pestle, 75-mm. outside diameter of mortar.

One portable gasoline stove.

Eight separatory funnels, Squibb type, 60-ml. capacity.

One separatory funnel rack.

Two serological pipets, 10-ml. capacity, calibrated in 0.1 ml.

Sieve, 80 mesh. Silk bolting cloth of 80 mesh in an aluminum holder, with 100-mm. outside diameter.

Stevens Extraction Sticks. These are made by constricting a glass tube, 170 mm. long and 7 mm. in inside diameter, near one

end, and packing the resulting small bulb with fine borosilicate glass fiber to serve as a filtering medium. Small cork stoppers can be inserted in the opposite end or a No. 13 ground-glass joint provided with a No. 13 glass stopper can be fused on that end. In the niobium method, corks are satisfactory.

One test tube rack, capacity of 20 tubes.

## PROCEDURE

**Solution of Samples.** Place 0.2 gram of soil or rock, ground to pass an 80-mesh sieve, and 4 grams of sodium bisulfate in a borosilicate glass culture tube. Mix by alternately rotating the tube and tapping gently against a hard surface. Place the tube in the fusion rack over the gasoline stove and heat to fuse the contents. Continue the fusion for 15 minutes. Remove the tube from the heat and rotate while cooling in order to form a thin coating of the fusion product around the tube walls. Add 10 ml. of $1M$ tartaric acid and insert a corked Stevens extraction stick, fitted with glass filtering fiber. Without heating the solution, use the extraction stick to break up the fused mass. If convenient, fuse samples in the afternoon, add the tartaric acid, and allow to stand overnight. When the fused mass is broken up, place tube and contents in a boiling water bath for 2 to 3 minutes. Remove the tube from the water bath and allow to cool. As the tube cools, the tartaric acid extract filters into the extraction stick.

**Development of Niobium Thiocyanate Complex.** Transfer a 1-ml. or a 2-ml. aliquot of the clear filtrate to a separatory funnel and add 5.0 ml. of hydrochloric acid–tartaric reagent to the 1-ml. aliquot or 5.6 ml. of the reagent to a 2-ml. aliquot. Shake and cool the contents of the separatory funnel to 20° or 25° C. Add 5 ml. of ammonium thiocyanate solution, and within 5 minutes add 5 ml. of ethyl ether reagent to the funnel. Shake the contents for 30 seconds. When the phases have separated, drain off the aqueous phase and add 2 ml. of stannous chloride reagent to the funnel. Shake for 10 seconds and allow the phases to separate. Drain and repeat the extraction with 2 ml. of stannous chloride reagent. If large amounts of iron(III) (red color in ether layer) or titanium(IV) are present, extract a third time with stannous chloride reagent. Transfer the ether phase to a 10-ml. volumetric flask, add acetone to the mark, and mix.

**Estimation.** If photometric equipment is available, transfer the ether acetone solution of niobium thiocyanate to a cuvette and measure the absorbance at 385 m$\mu$. From a previously established standard curve, determine the number of micrograms of niobium in the aliquot. Multiply by 50 if a 1-ml. aliquot (0.02 gram of sample) or by 25 if a 2-ml. aliquot had been taken (0.04 gram of sample) to obtain parts per million of niobium in the sample.

The estimations for all of the data given in this paper were made with photometric equipment.

Alternatively determine the niobium content of the sample solution as follows. Transfer a 10-ml. portion of the ether-acetone solution of the niobium thiocyanate complex to small flat-bottomed Nessler tubes and compare visually with standard ether-acetone solutions of niobium thiocyanate prepared by the foregoing procedures and containing, respectively, 10, 20, and 30 $\gamma$ of niobium. If the color intensity of the ether-acetone extract obtained from a sample solution is greater than that of the highest standard, dilute the extract obtained from the sample with a 1 to 1 mixture of ether and acetone, until its intensity is similar to one of the standards, and thus obtain an approximate value. Multiply the number of micrograms of niobium in the comparable standard by the ratio of the final volume of ether acetone to the original volume (10 ml.). Multiply the results by 50 or 25 as directed to convert to parts per million.

**Effectiveness of Sample Decomposition.** To gain some idea of the effectiveness of the sample decomposition, a sample of columbite was fused. Columbites are more or less soluble in molten alkali pyrosulfates (6), and the authors observed that a single fusion with 4 grams of flux dissolved more than 90 mg. of niobium.

Duplicate samples of a complex material, an igneous rock containing 10,000 p.p.m. of niobium, were fused by the recommended procedure, and the niobium contents were determined in the usual manner. The quantities found were, respectively, 8000 and 7000 p.p.m. The residues from the first fusion were fused again with the pyrosulfate flux, and the amounts of niobium present were determined and were found to be in both cases 1800 p.p.m. of niobium based on the original samples. The sums of the amounts of niobium found from the two fusions are 9800 and 8800 p.p.m.

The above experiments indicate that with complex materials repeated fusions result in larger recoveries, hence greater accuracy. Repeated fusions, being time-consuming, defeat the main objective of this work—the development of a rapid field method,

and should be omitted whenever possible. Because a single fusion brings into solution a major portion of the niobium present, the greater recovery achieved by repeated fusions is not considered sufficient to justify the additional time required. However, if greater accuracy is needed, some revision in the preparation of the sample solution is in order.

**Repeatability of Method.** To determine the repeatability of the proposed method, five separate niobium determinations were made at random on nine materials, comprising nepheline syenites and composite bauxites. The values obtained by each run, as well as certain derived data, are shown in Table V. The confidence limits at the 95% level are calculated as suggested by Youden (16).

The comparison of results obtained by a proposed method with those obtained by a recognized procedure constitutes a valid test of a method. Such a comparison is more rigorous, if the recognized procedure is independent of the proposed method. This condition was fulfilled for the authors' particular method by comparing results obtained by the proposed colorimetric method with those obtained by a quantitative spectrographic procedure. The results are shown in Table VI.

### Table VI. Determination of Niobium in Rocks

| Sample No. | Material | Niobium, P.P.M. | |
| --- | --- | --- | --- |
| | | Spectrographic[a] | Colorimetric |
| 1 | Nepheline syenite[b] | 60 | 70 |
| 2 | Nepheline syenite[b] | 70 | 90 |
| 3 | Nepheline syenite[b] | 80 | 100 |
| 4 | Nepheline syenite | 600 | 500 |
| 5 | Uraniferous monazite | 1100 | 1600 |
| 6 | Composite bauxite | 10000 | 9000 |

[a] Analyses by Paul Barnett, U. S. Geological Survey.
[b] Mineral separates.

The proposed method is relatively simple, rapid, and moderately accurate. Although an instrument is desirable to measure the absorbance of the niobium thiocyanate at 385 m$\mu$, it is not essential. The alternative method of estimation in which standards are prepared and comparisons made visually can be used in the field to obtain approximate results. A recent field application proved useful in guiding a large scale soil and rock sampling program. When the method is used routinely, more than 30 rocks can be analyzed for niobium in an ordinary 8-hour day. The moderate accuracy of the proposed method has been found to be sufficient for geochemical prospecting and other basic studies.

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# Powder Diffraction Standards for Niobium Pentoxide and Tantalum Pentoxide

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A re-examination of powder diffraction data for tantalum pentoxide has disclosed that niobium and tantalum were the chief constituents of the commercial sample used in the original investigation. New data have been obtained with tantalum pentoxide prepared by direct oxidation of tantalum metal and with a commercial sample of niobium pentoxide.

IN 1953 H. O. Spauschus informed the authors that standard pattern 905 (4) differed from the powder data of the tantalum pentoxide samples secured by him. The powder specimen of tantalum pentoxide used for pattern 905 was obtained from Eimer and Amend and was labeled "Tantalum Pentoxide, Acid Tantalic Anhydride, C.P." A rerun of the original sample confirmed the published diffraction data. However, a microcombustion of the same powder sample showed that it lost 6.5% by weight and contained 2.33% carbon and 0.70% hydrogen. Spectroscopic analysis of the ignited sample (giving the same powder pattern as the original material) showed tantalum and niobium as chief constituents and silicon, aluminum, and titanium as minor impurities. In view of these findings and the subsequent literature data (1–3, 5, 6), the authors prepared tantalum pentoxide by the direct oxidation of c.p. tantalum metal pur-

chased from the Fansteel Metallurgical Corp. The weight increase due to oxidation of the metal was determined:

In oxygen at 800° C.—21.92% (22.11% theory)
In air at 750° C.—21.97% (22.11% theory)

### Table II. Powder Diffraction Pattern of Pseudohexagonal Niobium Pentoxide[a]

| d, A. | [hkl] | (I/I₁)f | (I/I₁)i | B |
|---|---|---|---|---|
| 3.925 | 001 | 0.90 | 0.66 | 0.25° |
| 3.124 | 100 | 1.00 (125) | 1.00 | 0.48 |
| 2.446 | 101 | 0.40 | 0.39 | 0.42 |
| 1.962 | 002 | 0.30 | 0.18 | 0.35 |
| 1.800 | 110 | 0.25 | 0.30 | 0.86 |
| 1.663 | 102 | 0.30 | 0.21 | 0.49 |
| 1.637 | 111 | 0.14 | 0.15 | 0.75 |
| 1.565 | 200 | 0.12 | 0.08 | 0.8 |
| 1.456 | 201 | 0.08 | 0.07 | 0.8 |
| 1.327 | 112 | 0.18 | 0.10 | 0.8 |
| 1.309 | 003 | 0.04 | | |
| 1.222 | 202 | 0.08 | | |
| 1.205 | 103 | 0.06 | | |

[a] Filtered CuKα radiation employed to obtain powder diffraction data. d = interplanar spacing. (I/I₁)i = relative integrated intensity from Norelco diffractometer data. (I/I₁)f = relative intensity from film measured with intensity scale; wedge mounting. B = observed angular breadth of diffraction line at half-peak intensity. Lattice constants for pseudohexagonal cell containing 0.5 molecule of niobium pentoxide are: c = 3.925 A. and a = 3.607 A.

### Table I. Powder Diffraction Data for Niobium Pentoxide and Tantalum Pentoxide[a]

| Nb₂O₅ | | | Ta₂O₅ | | | Nb₂O₅ | | | | Ta₂O₅ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| d, A. | I/I₁ | [hkl] | d, A. | I/I₁ | | d, A. | I/I₁ | [hkl] | | d, A. | I/I₁ |
| | | 1̄21 | 5.29 | 0.03 | | 2.591 | 0.02 | 2̄04 | | | |
| 5.19 | 0.04 | 1̄02 | | | | | | 201 | | | |
| | | 101 | 4.295 | 0.01 | | | | 052 | | | |
| | | (1̄22) | | | | | | 2̄14 | | 2.535 | 0.04 |
| 3.924 | 1.00 | 040 | 3.885 | 1.00 | | | | 211 | | | |
| | | | | | | | | 151 | | | |
| 3.482 | 0.03 | 1̄13 | 3.485 | 0.01 | | | | | | | |
| | | 2̄12 | | | | 2.456 | 0.40 | 2̄43 | | 2.447 | 0.63 |
| | | 2̄11 | | | | | | 240 | | | |
| | | 131 | 3.338 | 0.05 | | | | 1̄61 | | | |
| | | 1̄40 | | | | | | | | | |
| | | 1̄41 | | | | 2.427 | 0.18 | 043 | | 2.420 | 0.36 |
| | | | | | | | | (3̄02) | | | |
| 3.146 | 1.00 | 200 | 3.150 | 1.00 | | | | 1̄34 | | 2.372 | 0.04 |
| | | 2̄03 | | | | | | 3̄13 | | | |
| 3.086 | 0.40 | 003 | 3.093 | 0.48 | | 2.121 | 0.04 | 212 | | | |
| | | (210) | | | | | | 2̄15 | | | |
| | | (2̄13) | | | | | | 2̄62 | | 2.101 | 0.01 |
| | | 042 | 2.984 | 0.02 | | | | 2̄61 | | | |
| | | 1̄51 | 2.860 | 0.02 | | | | 300 | | | |
| | | 122 | | | | | | 1̄63 | | | |
| | | 023 | | | | | | | | | |
| | | | | | | 2.033 | 0.02 | | | 2.02 | 0.03B |
| 2.725 | 0.03 | 2̄33 | 2.720 | 0.03 | | 2.007 | 0.07 | | | 1.945 | 0.20 |
| | | 230 | | | | 1.966 | 0.24 | | | 1.830 | 0.23 |
| | | 150 | | | | 1.872 | 0.01 | | | 1.800 | 0.12 |
| | | 033 | 2.648 | 0.03 | | 1.845 | 0.03 | | | 1.765 | 0.04 |
| | | 1̄43 | | | | 1.827 | 0.15 | | | 1.678 | 0.01 |
| | | 132 | | | | 1.791 | 0.20 | | | 1.655 | 0.36 |
| | | 2̄42 | | | | 1.734 | 0.01 | | | 1.631 | 0.04 |
| | | 2̄41 | | | | 1.696 | 0.01 | | | 1.606 | 0.03 |
| | | | | | | 1.666 | 0.18 | | | 1.577 | 0.04 |
| | | | | | | 1.655 | 0.15 | | | 1.549 | 0.04 |
| | | | | | | 1.626 | 0.11 | | | 1.497 | 0.03 |
| | | | | | | 1.606 | 0.01 | | | 1.462 | 0.08 |
| | | | | | | 1.572 | 0.08 | | | 1.440 | 0.03 |
| | | | | | | 1.539 | 0.03 | | | 1.405 | 0.03 |
| | | | | | | 1.517 | 0.01 | | | 1.386 | 0.03 |

[a] Filtered CuKα radiation was used to obtain the powder data. d = interplanar spacing. I/I₁ = relative peak intensity. [hkl] are the reflection indices. For niobium pentoxide the cell dimensions containing 12 niobium pentoxide molecules are: a = 7.31 A., b = 15.72 A., c = 10.75 A., β = 120° 42'; for tantalum pentoxide, a = 7.32 A., b = 15.55 A., c = 10.79 A., β = 120° 36'.

To confirm that the resultant powder data from this sample of tantalum pentoxide did not correspond to a polymorphic mixture, an isomorphous niobium pentoxide was prepared, and the two patterns were indexed on the basis of the smallest unit cell accounting for the observed reflections. The "low" temperature modification (5) of tantalum pentoxide and niobium pentoxide of Table I may well be orthorhombic instead of monoclinic. The monoclinic cell is derived as a distortion of the pseudohexagonal modification of niobium pentoxide and is related structurally to columbite (7). Table II contains the diffraction data for the pseudohexagonal modification of niobium pentoxide purchased from A. D. Mackay, Inc. Spectroscopic examination of this material proved it to be free of tantalum and revealed the following impurities in concentrations of 10 to 100 p.p.m.: calcium, copper, magnesium, and silicon (no hydrogen determination was made). On heating this high-area oxide (34 sq. meters per gram) for 16 hours at 700° C., it was converted to

the "low" modification of Table I. The pseudohexagonal modification of tantalum pentoxide has the cell dimensions $a = 3.60 \pm 0.02$ A., $c = 3.88 \pm 0.01$ A.

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# Rapid Determination of Cobalt in Alloy Steels by the Tetraphenylarsonium Method

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The usual methods for the determination of cobalt in stainless steels and similar materials are wanting in several respects. A slight modification of rapid, precise colorimetric method, originally described by Potratz and coworkers, is presented. The procedure is designed for stainless steels but is easily adapted for other materials. A mean deviation for precision of ±0.001% is attained.

THE methods for determination of cobalt in steels (2, 3) are wanting in several respects in ordinary steels and even more so in alloy steels. In order to obtain adequate precision, such methods as the umpire electrolytic method are very long and involve so many manipulatory steps that the possibility of errors and associated lack of precision is very large. Furthermore, the conversion to the final weighing form is often open to question. The exact formula of the cobaltic oxide ($Co_3O_4$) usually used is in doubt, as it may contain cobalt(II) oxide (CoO). Similar difficulties arise with the sulfate, and the reduction to metal is an added step when time is important.

### Table I. Recovery of Cobalt from NBS Samples

| Sample Designation | Certificate Value, % | No. of Determinations | Mean Value, % | Mean Deviation % |
|---|---|---|---|---|
| 101d | 0.056a | 2 | 0.056 | 0 |
| 126A | 0.30 | 12 | 0.30 | ±0.001 |
| 153 | 8.45 | 3 | 8.29 | ±0.01 |
| 161 | 0.47 | 4 | 0.47 | 0 |
| 169 | 0.19 | 4 | 0.19 | ±0.001 |

a Provisional value.

Colorimetric methods, such as the nitroso-R procedure, are lacking in precision because of the many interferences (1, 5) such as copper, nickel, iron, chromium, manganese, and nitrates. These substances must be removed for satisfactory results, and the added separation steps materially decrease the precision and lengthen the time required.

Potratz and coworkers have recently described a very satisfactory colorimetric method (1, 4) using the tetraphenylarsonium cobaltothiocyanate complex for the determination of cobalt. The procedure is rapid and precise, being equally as good as the usual reference method. Furthermore, much smaller concentrations of cobalt than usually required can be conveniently measured by merely increasing the sample size without altering the described procedure. Interference by elements such as iron, copper, molybdenum, and vanadium is eliminated or minimized by reduction and complexing (1).

The prime interest in this laboratory has been in stainless steels of the 18 8 type, and because these alloy steels cause the most

difficulty in analysis, the procedure developed is described for this material. Some minor changes have been made in the original Potratz procedure. The analysis of other materials differs only in the original solution step, which can usually be simplified.

### PROCEDURE

Dissolve a 0.5- to 1.0-gram sample in aqua regia by heating gently until only the carbides and silica remain as the precipitate. Dilute the acid solution with 25 ml. of distilled water, and filter through a coarse paper into a 200-ml. volumetric flask. Wash the paper a few times with 2% hydrochloric acid and then thoroughly with water.

Dilute to volume, mix thoroughly, and transfer an aliquot containing 50 to 150 γ of cobalt to a 150-ml. beaker. Evaporate to dryness without baking. Cool, dissolve the residue in a few drops of concentrated hydrochloric acid, and again evaporate to dryness to remove most of the nitrate.

Dissolve the residue in a minimum amount of 1 to 1 hydrochloric acid, dilute with about 20 ml. of water, and neutralize with 1 to 1 ammonium hydroxide. The final solution should be just acid.

Add successively with stirring 3 to 4 drops of freshly prepared 10% potassium thiosulfate, 10 ml. of 50% ammonium thiocyanate, and 1.5 grams of ammonium fluoride. These reagents will complex the interfering substances such as iron and form the cobaltothiocyanate complex. With stainless steels, the use of thiosulfate and the larger fixed quantity of fluoride materially increased the stability of the color.

Transfer this solution quantitatively to a 150-ml. separatory funnel and add 15 drops of a 0.05M tetraphenylarsonium chloride solution. (The tetraphenylarsonium chloride reagent can be procured from Hach Chemical Co., Ames, Iowa, Eimer and Amend, New York, N. Y., or A. D. Mackay, Inc., New York, N. Y.) Extract with 9 ml. of chloroform by shaking vigorously for 1 minute. Filter the chloroform layer through a cotton pad which has previously been rinsed with chloroform into a 25-ml. volumetric flask. Repeat the extractions twice more using 5-ml. portions of chloroform and 5 drops of the tetraphenylarsonium solution. Dilute to volume with chloroform.

Measure the absorbance of the solution at 620 mμ using a 25-mm. cuvette. Correct this value by determining the reagent blank in a similar manner; it has been the authors' exeprience that a relatively high blank will occur with some lots of ammonium fluoride.

Standard curves can be prepared from spectrographically pure cobalt metal or by the gravimetric standardization of cobalt(II) nitrate hexahydrate [$Co(NO_3)_2.6H_2O$]. In either case before the stock solution is prepared, nitrate should be removed by fuming with sulfuric acid.

### RESULTS

The accuracy of the method has been amply demonstrated by Affsprung, Barnes, and Potratz (1), who obtained excellent results with National Bureau of Standard steels. The data given in Table I indicate the good precision obtainable.

Despite the disagreement of the value obtained by the method described with the NBS value for sample 153, the precision obtained is good. On a relative basis the values checked to 1 part

per thousand. A number of other cooperative samples have been analyzed in a standards certification program with the National Bureau of Standards and other laboratories with excellent checks and equally good precision.

## CONCLUSION

The tetraphenylarsonium method described by Potratz and coworkers is highly recommended for the determination of cobalt in stainless steels and other ferrous samples. The method is rapid and precise, being equally as good as the usual reference procedure. Exceptionally good precision is obtained and the procedure has been applied to a wide variety of materials such as the Inconels, nickel, chromium, and boron carbide.

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# Volumetric Determination of Mercury and the Use of Mercury Salts as Primary Acidimetric Standards

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A rapid and accurate volumetric method for the determination of mercury consists of converting the mercury into neutral mercury(II) oxide, which is held in solution by complexing with a small quantity of acetamide or a large quantity of urea. The mercury is estimated by the acidimetric determination of the alkali liberated when potassium iodide or sodium thiosulfate is added to this mercury(II) oxide complex. Because mercury(I) ion disproportionates quantitatively into mercury(II) ion and metallic mercury when alkali is added in the presence of an amide, it can be estimated by determining the mercury(II) ion in solution after such disproportionation using the method described. The accuracy with which mercury can be estimated with this method has prompted the recommendation of mercury salts as reliable primary acidimetric standards.

$D$AS (1), working in this laboratory, has developed a method for alkalimetric estimation of mercury utilizing the well known solubility of mercury(II) oxide in acetone. The method consists of conversion of the mercury(II) salt into mercury(II) oxide, which remains in solution in presence of acetone, followed by the liberation of an equivalent quantity of alkali by a large excess of potassium iodide or sodium thiosulfate ($HgO + 4KI = K_2HgI_4 + 2KOH$).

The method gives excellent results in favorable cases but has a number of drawbacks: It is not applicable in the presence of a few interfering ions such as halide and phosphate. A precipitate of basic mercury(II) salt is sometimes formed on addition of alkali or acetone, and this often takes an inordinately long time for complete dissolution in the aqueous acetone medium. The amount of potassium iodide or sodium thiosulfate added to liberate the alkali is required in large excess of the theoretical amount, and the amount of acetone required is much greater than the amount of mercury present. The present paper describes an improved method in which all the above drawbacks have been almost completely eliminated, and it has been possible to use mercury(II) salts as primary standards in acidimetry.

## THEORETICAL

The method depends on the fact that any amide can prevent precipitation of mercury(II) oxide when strong alkali is added to a mercury(II) salt. The amide can be present only in a very small quantity—for example, a few milligrams of acetamide or formamide (0.5 mole of the former or 1 mole of the latter per mole of mercury(II) oxide) are sufficient to prevent precipitation of mercury(II) oxide from 10 ml. of $0.1N$ mercury(II) salt solution. Though any unsubstituted amide or imide would presumably be suitable, for various reasons acetamide or urea has been chosen for the present method, even though the latter is required in a comparatively large excess. The present method is similar to that of Das, except that a slight excess of acetamide or a large excess of urea (roughly 20 times the weight of mercury) is used in place of acetone to keep the mercury(II) oxide in solution. Urea or acetamide is far superior to acetone, as not a trace of solid is precipitated by an excess of alkali and a bare excess of iodide or thiosulfate is required in the second stage. The method gives excellent results when no interfering anions such as chloride or phosphate are present.

In the presence of chloride ions, which is rather common, the first end point is much less sharp than is usual in strong acid–strong base titrations owing to a progressive liberation of alkali by the chloride. Das has recommended using a mixed indicator which, though slightly better, falls short of the ideal. The difficulty, however, has been completely surmounted by conducting the first neutralization with alcoholic alkali in a 75% ethyl alcohol medium when the end point becomes very sharp. The second titration, after liberating the alkali by iodide or thiosulfate, is extremely sharp in all cases.

## GENERAL PROCEDURE

**In Absence of Interfering Ions.** To about 20 ml. of a solution containing about a millimole of mercury are added 4 to 5 grams (about 20 to 25 times the weight of mercury) of urea and a few drops of phenolphthalein indicator solution. Alternatively, 70 mg. of acetamide (5 ml. of a freshly prepared 1.4% solution) per millimole of mercury can be used in place of urea. The solution is now made alkaline by adding a slight excess of dilute sodium hydroxide solution. A dilute acid solution (roughly $0.1N$ perchloric acid or nitric acid) is added drop by drop from a buret to a sharp decolorization of the phenolphthalein. About 12 ml. of a neutral (roughly $0.5N$) solution of potassium iodide or sodium thiosulfate (10 to 20% in excess of the theoretical) is now added, when the solution again becomes vividly pink owing to liberation of alkali. The solution is titrated against a standard $0.1N$ acid to an extremely sharp phenolphthalein end point. Hydrochloric acid is undesirable for the preliminary neutralization owing to interference by the chloride ion. However, in the final titration with the standard acid all acids, including weak acids such as acetic acid, are suitable.

Some typical results are given in Table I for salts prepared from analytical grade mercury(II) oxide, as they are not available in an analytically pure state. Mercury(II) sulfate is available in analytically pure quality and can be estimated by the above procedure, provided it can be brought in solution. To facilitate dissolving, 1 or 2 millimoles of mercury(II) sulfate are treated with a slight excess of 0.1N alkali followed by 20 ml. of a 25% urea solution. On gentle warming all the mercury(II) sulfate is dissolved and is ready for the usual analysis.

Urea and acetamide have both been used in the proportions mentioned above, and have been found to give equally satisfactory results. Potassium iodide and sodium thiosulfate function equally satisfactorily; potassium bromide can also be used with complete success, provided an excess of about twice the weight of mercury present is used.

Attempts have been made to dispense with iodide or thiosulfate by using a double indicator method. The accuracy attainable is very low, owing to a very gradual change of pH, as ascertained by potentiometric titration, if further acid is added after discharge of the phenolphthalein color.

**Direct Titration with Alkali.** As the first end point with alkali is sharp, it seems that a direct titration with alkali is possible. In fact, Fernandez and coworkers (2) have described such a method using acetone as the complexing agent. An investigation of this possibility was not made with the present method, as such a method would not have many applications—most mercury salts available are stoichiometrically inexact, and several of them require the addition of acid for their dissolution. However, a solution of mercury(II) acetate obtained by dissolving the salt in water and filtering out the small amount of undissolved residue gives almost identical values by direct alkali titration and by acid titration as described previously.

**Table I.  Estimation of Mercury(II) Salts Using Standard Nitric Acid**

| Anion | HgO, Gram | | Error, % |
|---|---|---|---|
| | Taken | Found | |
| Acetate | 0.0504 | 0.0504 | 0.00 |
| | 0.1328 | 0.1329 | +0.07 |
| | 0.2764 | 0.2763 | −0.04 |
| Nitrate | 0.1064 | 0.1064 | 0.00 |
| | 0.1765 | 0.1766 | +0.06 |
| | 0.3396 | 0.3398 | +0.06 |
| Perchlorate | 0.0501 | 0.0500 | −0.20 |
| | 0.1234 | 0.1234 | 0.00 |
| | 0.2663 | 0.2660 | −0.11 |
| Sulfate | 0.0809 | 0.0808 | −0.12 |
| | 0.1458 | 0.1456 | −0.14 |
| | 0.4023 | 0.4019 | −0.10 |

For other salts such as sulfate and nitrate, the alkali titration values are slightly higher, presumably owing to some basic salt remaining undissolved on treatment with water. The discrepancy between the acid and the alkali titer of a mercury salt can be used as a measure of the stoichiometric inexactness of the salt.

**Interfering Ions Present.** In the presence of other cations the mercury can be separated quantitatively by methods similar to those outlined by Das, except that acetamide or urea is used in place of acetone. The substitutions have the advantage of avoiding the occasional precipitation of basic mercury(II) salt.

Of the interfering anions—halide, thiocyanate, thiosulfate, cyanide, and phosphate—chloride ions are common, and the following modification works satisfactorily in their presence. The method is illustrated by an analysis of mercury(II) chloride which is available in the analytical reagent grade.

About 0.2 gram of mercury(II) chloride is dissolved in 20 ml. of a 1 to 3 mixture of water and alcohol (or rectified spirit). After all the mercury salt is dissolved (1 or 2 minutes), 5 grams

of urea and 3 drops of 1% phenolphthalein solution are added to the solution. The solution is now made slightly alkaline with alcoholic sodium hydroxide, and a dilute acid solution is added dropwise from a buret until the alkaline color just disappears. Ten milliliters of neutral (approximately 0.5N) potassium iodide or sodium thiosulfate solution are added and the liberated alkali is titrated against a standard acid to an extremely sharp phenolphthalein end point. Methanol can be used with equal success in place of ethyl alcohol in this titration. Sometimes a white precipitate forms on addition of urea to solutions containing mercury(II) salts. This is generally due to an insufficiency of urea and can be rectified by adding more.

Some typical results are shown in Table II which indicate the precision of the method.

**Table II.  Estimation of Mercury(II) Chloride**

| Standard Acid | Taken, Gram | Found, Gram | Error, % |
|---|---|---|---|
| Perchloric (Na₂S₂O₃ used) | 0.0909 | 0.0908 | −0.11 |
| | 0.1787 | 0.1790 | +0.17 |
| | 0.2256 | 0.2256 | 0.00 |
| | 0.3359 | 0.3360 | +0.03 |
| Nitric (KI used) | 0.1366 | 0.1368 | +0.15 |
| | 0.1794 | 0.1794 | 0.00 |
| | 0.1830 | 0.1834 | +0.22 |
| | 0.4816 | 0.4817 | +0.02 |
| Hydrochloric | 0.1863 | 0.1860 | −0.16 |
| | 0.2466 | 0.2471 | +0.20 |
| | 0.3208 | 0.3216 | +0.25 |
| Acetic | 0.1085 | 0.1083 | −0.18 |
| | 0.1917 | 0.1915 | −0.11 |
| | 0.2995 | 0.2998 | +0.10 |

**Table III.  Estimation of Mercury(II) and Mercury(I) Sulfate Using Standard Nitric Acid**

| Compound | Taken, Gram | Found, Gram | Error, % |
|---|---|---|---|
| Mercury(II) sulfate | 0.0906 | 0.09075 | +0.16 |
| | 0.1148 | 0.1147 | −0.08 |
| | 0.1181 | 0.1183 | +0.17 |
| | 0.2042 | 0.2037 | −0.25 |
| Mercury(I) sulfate | 0.1764 | 0.1764 | 0.00 |
| | 0.1798 | 0.1801 | +0.17 |
| | 0.2021 | 0.2020 | −0.05 |
| | 0.2161 | 0.2162 | +0.05 |

Phosphate can be removed easily by carrying out the preliminary neutralization with barium hydroxide in place of sodium hydroxide until all the phosphate is precipitated. The filtrate can then be titrated as usual. This is an improvement over the method used by Das which involves two steps: removal of the phosphate by an excess of barium nitrate and subsequent removal of the latter as sulfate.

The interference by chloride is much less than that by bromide or iodide, and with a little practice fairly accurate values can be obtained by utilizing the normal procedure, particularly if a mixed indicator—phenolphthalein (3 parts) and 1-naphtholphthalein (1 part)—is used as recommended by Das.

### INTERACTION BETWEEN AMIDE AND MERCURY

That mercury(II) oxide is not precipitated in the presence of a variety of substances including amides is well known and is referred to by Mellor (9) and Sidgwick (11). According to Schoeller and Schrauth (10), mercury(II) oxide dissolves in a solution of an amide such as acetamide to give (CH₃CO NH)₂ Hg; imides, especially cyclic imides such as succinimide, react very easily. Ley and others (6-8) have shown that the complex is only slightly ionized and reacts only slowly with potassium iodide. Sidgwick (12) mentions that the mercury-benzamide complex has the formula (C₆H₅CONH)₂ Hg and is so stable that it can be crystallized from hot potassium hydroxide solution without decomposition. The formation of a urea–mercury(II) nitrate com-

plex is also well known, and it has the formula OH.HgNHCONH-Hg.NO₃ (3).

Though the isolated compounds may have the above formulas, it has been observed that 1 mole of acetamide can prevent precipitation of 2 moles of mercury and formamide is only roughly half as powerful, whereas dimethylformamide is practically devoid of any such power. Compounds of the types CH₃CON-(HgOH)₂ and HCONHHgOH are apparently formed, if only in solution. It is also possible to write six-membered cyclic donor-acceptor structures:



I                                    II
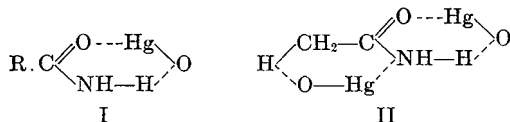
Such formulas merit serious consideration in view of the unique properties of mercury—e.g., mercuration, addition to double bond—as contrasted to any other metal.

### ESTIMATION OF MERCURY(I) SALTS

Mercury(I) salt can be estimated by the present method because on addition of alkali and amide to a mercury(I) salt in solution as well as in the solid state, mercury(I) disproportionates into metallic mercury and mercury(II) ions. The black precipitated mercury can be redissolved and analyzed by the present procedure or the mercury(II) ion remaining in solution can be thus analyzed.

The above procedure has been tested by using analytically pure mercury(I) sulfate. The latter is dissolved by treating 1 millimole with a slight excess of 0.1N sodium hydroxide solution followed by 10 ml. of 25% urea solution. The solution is warmed for a few minutes to complete the reaction. The black precipitate of mercury which settles down is filtered off and washed out, and the filtrate is analyzed volumetrically for the mercury(II) salt. The results are shown in Table III. Mercury(I) chloride is also dissolved by the same method, but the filtrate contains chloride ion and so an acid titration by the usual method using the mixed indicator in place of phenolphthalein is possible but yields somewhat less accurate results. The residue of mercury can be dissolved and titrated, but that method has not been tested.

### MERCURY SALTS AS PRIMARY STANDARDS IN ACIDIMETRY AND ALKALIMETRY

From the high degree of accuracy attainable with the present method of estimating mercury it is evident that if mercury salts of the analytical reagent grade which are stable and stoichiometrically exact could be obtained, they could be utilized for standardization of both acids and alkalies. This method, unlike that involving the use of carbonates and borax (Na₂B₄O₇·10H₂O), would require few special precautions.

For standardizing strong alkali a known amount of the mercury salt in the absence of interfering ions could be titrated against the alkali using phenolphthalein as indicator in the presence of urea or acetamide. Alcoholic alkali could be conveniently standardized using mercury(II) chloride. For standardizing an acid, the alkali liberated by the addition of a slight excess of iodide or thiosulfate during the second stage of this method of estimating mercury could be titrated.

Unfortunately stoichiometrically exact and analytically pure mercury salts are not available except mercury(II) chloride, mercury(II) sulfate, and mercury(I) sulfate. Mercury(II) chloride could be used for standardizing an acid by the special method of using alcoholic medium as already described. Mercury(II) sulfate and mercury(I) sulfate are satisfactory for the purpose, as they could be used by following the usual procedure after dissolving them by methods already described.

To test the theory, four acid solutions—nitric, perchloric, hydrochloric, and acetic—were standardized against analytical

grade mercury(II) chloride, mercury(II) sulfate, and mercury(I) sulfate. Mercury(II) oxide, which has been recommended as a primary acidimetric standard, was used as a reference standard, the method having been checked by Kolthoff and van Berk (4). The results are shown in Table IV which shows that mercury salts are useful for standardizing both weak and strong acids.

**Table IV. Standardization of Acids Using Mercury(II) Oxide, Mercury(II) Chloride, Mercury(II) Sulfate, and Mercury(I) Sulfate**

| Acid | HgO, N | HgCl₂, N | HgSO₄, N | Hg₂SO₄, N |
|---|---|---|---|---|
| Nitric | 0.09790 | 0.09780 | 0.09779 | 0.09782 |
| Perchloric | 0.07626 | 0.07625 | 0.07608 | 0.07610 |
| Hydrochloric | 0.07630 | 0.07610 | ... | 0.07619 |
| Acetic | 0.1030 | 0.1030 | 0.1028 | 0.1030 |

Chloride ions interfere with the first neutralization but do not affect the final titration with acid; thus hydrochloric acid can also be standardized by this method. The most convenient method, however, for routine laboratory purpose is to use a stock solution of the easily dissolved mercury(II) acetate and use it as the routine alkali solution of known strength after standardizing it against a standard acid or potassium biphthalate solution. Such stock solutions seem to be indefinitely stable when kept in the dark. Use of a solution of sodium hydroxide which is free of carbonate and is stored in a "seasoned" bottle (5) would no doubt be satisfactory, but as no such special precautions need be taken with mercury salts the latter may be used with greater ease.

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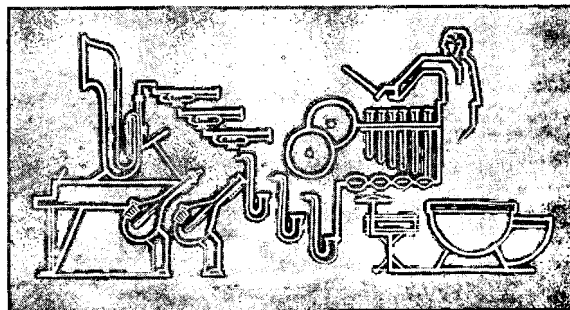
# Ultraviolet Photometric Titrations of Bismuth and Lead with Ethylenediaminetetraacetic Acid

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In titrations of bismuth with ethylenediaminetetra-acetic acid where indicators are used, interferences arise from the interaction of extraneous cations with the indicators. Indicator problems can be circumvented by photometric titrations based on the ultraviolet absorption of the bismuth–ethylenediamine tetraacetate complex. Although this method is subject to interferences of its own, it supplements other types of titrations with different interferences. Lead can be titrated similarly, and bismuth and lead can be determined in a single titration.

SEVERAL recent papers have described photometric titrations of various cations with ethylenediaminetetraacetic acid [(ethylenedinitrilo) acetic acid, Versene, EDTA], and the merits of this technique have been adequately reviewed (3–9). Of its advantages, perhaps the most attractive is the circumvention of some of the interferences and other difficulties attending the use of visual indicators for the titrations. In a previous paper (8), titrations of bismuth with ethylenediaminetetraacetic acid were described in which indication of the end points by cupric ion and by thiourea was facilitated by the photometric technique. It is now of interest to report photometric titrations in which the ultraviolet absorption by the bismuth complex of the titrant furnishes the only indicator necessary. Similar titrations are also possible with lead, and bismuth and lead can be determined simultaneously in a single titration.

## APPARATUS AND REAGENTS

The Beckman Model DU spectrophotometer, equipped with a hydrogen discharge tube, was adapted for the titrations (8). The titration cell was the same as that used before, except that the portion of the cell which was placed in the light path was fabricated from Vycor 7910 glass; this high-silica glass has a fairly large ultraviolet transmittance [70% at 254 mμ for a thickness of 2 mm. (2)]. The length of the light path through the solution was about 1.5 cm. Absorption spectra were obtained in the usual way, using 1-cm. silica cells. pH measurements were made with a Beckman Model G pH meter.

The disodium salt of ethylenediaminetetraacetic acid (Versenes, Inc., Framingham, Mass., disodium Versenate, analytical reagent) was dissolved in distilled water to prepare solutions of the titrant (generally 0.01 or 0.001M) which were standardized by photometric titrations against standard bismuth and lead solutions.

Standard solutions of bismuth and of lead were prepared from the pure metals. From the standpoint of noninterference in the ultraviolet region of the spectrum, perchlorate is a satisfactory anion to accompany these metals. The perchlorate solutions were prepared from nitric acid solutions of the metals by fuming with perchloric acid, followed by dilution to known volumes with distilled water.

In cases where buffering was necessary, chloroacetic acid was used as described previously (8). In most of the titrations reported here, the solutions were sufficiently dilute and special buffering was not required to maintain constant pH values during the titrations.

All other materials were reagent grade or the equivalent.

## ABSORPTION SPECTRA

Bismuth and lead ions, and their ethylenediamine tetraacetate complexes, are colorless. However, they all exhibit strong absorption bands in the ultraviolet region of the spectrum, as shown in Figure 1. The spectra shown here were obtained in perchlorate solutions of pH 2. The absorption peaks of lead and

bismuth ions are shifted by numerous anions—nitrate, for example—presumably through the formation of complex ions. The spectra of the complexes are not affected in this way, because these complexes are much more stable than those formed with ordinary anions. The absorption maximum of the lead complex is at 240 mμ, and for the bismuth complex at 265 mμ.
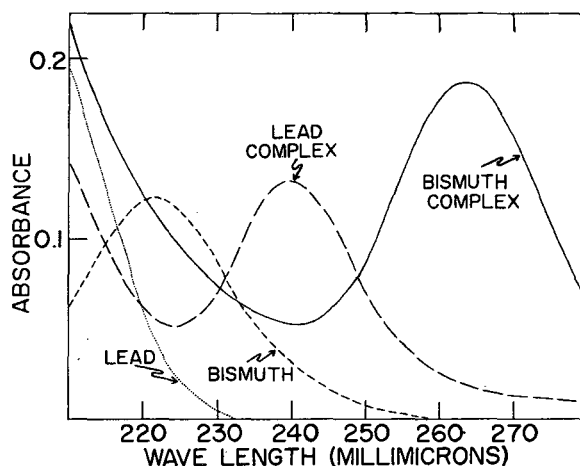


**Figure 1. Absorption spectra of bismuth and lead perchlorates and their ethylenediaminetetraacetic acid complexes**

$2 \times 10^{-5}\ M$ Bi and Pb in $10^{-2}\ M$ HClO complexes at same concentration

These spectra are very favorable for the present purpose. Many of the metal ion complexes (and ethylenediaminetetraacetic acid itself) absorb only near the lower wave length limit of the Beckman Model DU instrument; it is difficult to perform photometric titrations at these short wave lengths because of the strong absorption by many extraneous ions. As shown in Figure 1, the spectrum of the bismuth complex is more favorable than that of the lead complex in this regard. As lead often occurs in large quantities in samples where bismuth determinations are desired, it is fortunate that neither the lead ion nor its ethylenediamine tetraacetate complex absorbs appreciably in the region of the bismuth complex absorbance maximum.

## BISMUTH TITRATIONS

Figure 2 shows the photometric titration of a pure, $2 \times 10^{-4}M$ bismuth solution. Maximum sensitivity is obtained, of course, at the wave length (265 mμ) where the bismuth complex exhibits its absorption maximum. Since restriction to this wave length is obviously not obligatory, in general a wave length is selected at which absorbance readings will remain on scale throughout the titration. A very rough estimate of the bismuth concentration is sufficient to permit the selection of a suitable wave length. A pH value of about 2 is recommended for the bismuth titrations. The bismuth complex with ethylenediaminetetraacetic acid is sufficiently stable to be formed quantitatively at this pH. Successful titrations have been performed at pH 1.5; below this pH value, the end points become less distinct. The

tendency of bismuth to precipitate becomes troublesome at pH values greater than about 2.5. For bismuth solutions which are less concentrated than about $5 \times 10^{-4}M$, titrated with $10^{-3}M$ ethylenediaminetetraacetic acid, it is not necessary to buffer the solutions, because there is very little pH change during the titrations. Acid solutions of bismuth are merely adjusted to pH 2 with sodium hydroxide in such cases. When more concentrated bismuth solutions are titrated, pH changes during the titration result in less distinct end points unless the solutions are buffered.

### Table I. Titration of Pure Bismuth Solutions

| Bi Taken, Mg. | Bi Found, Mg. | Error, Parts/1000 |
|---|---|---|
| 0.209 | 0.213 | 20 |
| 0.209 | 0.198 | 55 |
| 0.209 | 0.209 | 0.0 |
| 1.05 | 1.07 | 20 |
| 1.05 | 1.04 | 10 |
| 2.09 | 2.19 | 48 |
| 2.09 | 2.08 | 4.8 |
| 4.18 | 4.16 | 4.8 |
| 4.18 | 4.08 | 23 |
| 4.18 | 4.18 | 0.0 |
| 5.22 | 5.24 | 3.8 |
| 6.27 | 6.26 | 1.6 |

A chloroacetate buffer, as used previously (8), is recommended for such cases. The end point shown in Figure 2 is seen to be very sharp, and satisfactory end points are obtained even with $10^{-6}M$ bismuth solutions. It appears that this lower limit might be extended by using a titration cell with a longer light path. Some analytical results obtained with pure bismuth solutions are given in Table I. In all cases, the volume was 100 ml.; such a volume is not necessary, of course, and hence very small quantities of bismuth (a few micrograms) could be determined by "scaling down" the procedure.

### LEAD TITRATIONS

The typical titration curve for lead has the same appearance as the bismuth curve shown in Figure 2. As noted above, the wave length for maximal sensitivity is 240 m$\mu$, although, as in the case of bismuth, the wave length of choice depends upon the concentration of the lead solution. The practical lower limit for the titration of pure lead solutions appears to be about $10^{-6}M$.

### Table II. Titration of Pure Lead Solutions

| Pb Taken, Mg. | Pb Found, Mg. | Error, Parts/1000 |
|---|---|---|
| 0.207 | 0.210 | 15 |
| 0.414 | 0.410 | 9.7 |
| 0.414 | 0.409 | 12 |
| 0.414 | 0.415 | 2.4 |
| 0.828 | 0.830 | 2.4 |
| 2.07 | 2.10 | 15 |

Typical results obtained with pure lead solutions are given in Table II. The titrations were performed at pH 2, although higher pH values would presumably be permissible, as the lead ion is not readily hydrolyzed.

### SIMULTANEOUS TITRATION OF BISMUTH AND LEAD

It has been pointed out (7–9) that if two metal ions differ sufficiently in the stability of their ethylenediamine tetraacetate complexes, and if the absorption spectra of the ions and their complexes are favorable, it is possible to obtain end points for the two metals in a single photometric titration with ethylenediaminetetraacetic acid. Bismuth and lead furnish another example of this technique, which is illustrated in Figure 3. The titration was performed at 240 m$\mu$. As the bismuth complex is much more stable than the lead complex, bismuth reacts first with the titrant. The titration curve rises slightly during the formation of the bismuth complex, because it absorbs somewhat more strongly than

the bismuth ion itself at 240 m$\mu$. After the bismuth has reacted essentially completely, the less stable lead complex begins to form, and the curve rises steeply because the lead complex absorbs much more strongly than the bismuth complex at this wave length. Finally, a plateau is reached, representing completion of the lead titration and reflecting the fact that the excess ethylenediaminetetraacetic acid does not absorb appreciably at 240 m$\mu$. In the present investigation, interest was centered on bismuth, and this simultaneous determination of bismuth and lead has not been thoroughly studied. The data shown in Figure 3 indicate, however, the possibility of developing useful applications.

### INTERFERENCES IN BISMUTH DETERMINATION

It is convenient to classify the interfering ions into two main groups. The first group is composed of those cations whose ethylenediamine tetraacetate complexes are sufficiently stable (relative to the bismuth complex) that the ions react with the titrant during the bismuth titration. The bismuth complex is so stable that very few ions interfere in this way. Ferric iron is one of the few in this class, and it causes high results in the bismuth determination in direct accordance with the quantity of iron present. Since the ferrous complex is much less stable than the bismuth complex, perhaps this interference could be circumvented by a simple reduction. Thorium is similar to ferric iron in its effect. It is fortunate that lead does not interfere, as lead frequently accompanies bismuth in alloys and other samples of practical interest. Copper, cobalt, nickel, cadmium, and zinc were found not to interfere unless present in massive quantities, in which case they caused drifting in the absorbance readings. This type of interference can be predicted from the formation constants of the ethylenediaminetetraacetic acid complexes of the ions in question. (In the titration of lead, the interference picture is not so favorable, as copper, nickel, cobalt, and several other metal ions form complexes which are about as stable as the lead complex.)
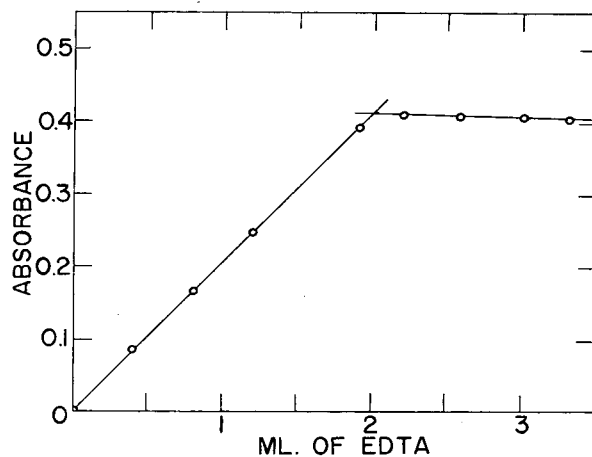


**Figure 2. Photometric titration of pure bismuth solution**

100 ml. of $2 \times 10^{-4}$ M titrated with $10^{-2}$ M ethylenediaminetetraacetic acid at 265 m$\mu$

The second type of interfering ions includes those which do not compete with bismuth for the ethylenediaminetetraacetic acid, but which cause difficulty in the spectrophotometry because they or their complexes absorb at the wave lengths used in following the bismuth titration. Moderate amounts of such extraneous absorption can be easily countered merely by using larger slit widths. Furthermore, the severity of this type of interference often depends upon the bismuth concentration, which dictates the sensitivity required and hence the choice of wave length. Thus, a

discussion of this problem is necessarily vague, the ultimate decision depending upon the composition of the particular solution with which one is working. The particularly troublesome absorption due to nitrate ion at 280 to 300 mμ should be noted. Perchlorate, sulfate, and chloride, on the other hand, do not interfere. The occasional interference of copper serves as an example of the care which must be taken in the selection of wave lengths. At 265 mμ, copper does not interfere, as stated above, but it was found troublesome in a titration at 285 mμ; at the latter wave length, the copper–ethylenediamine tetraacetate complex has almost the same absorptivity as the bismuth complex, and hence the titration curve, instead of becoming horizontal, shows only a small change in slope at the bismuth end point. Lead, cadmium, zinc, cobalt, and nickel ions were found not to absorb strongly enough either at 265 mμ or higher wave lengths to interfere in the bismuth titration.

Tin may be considered more or less by itself as an interfering ion. Because of its tendency to precipitate as the hydrous oxide, its behavior in the titration of bismuth has not been studied. Tin is conveniently eliminated by volatilization as stannic bromide according to the standard procedure (1), modified only by omitting phosphoric acid from the recommended solutions. As this procedure also eliminates arsenic and antimony, the interference of these elements in the bismuth titration has not been investigated.

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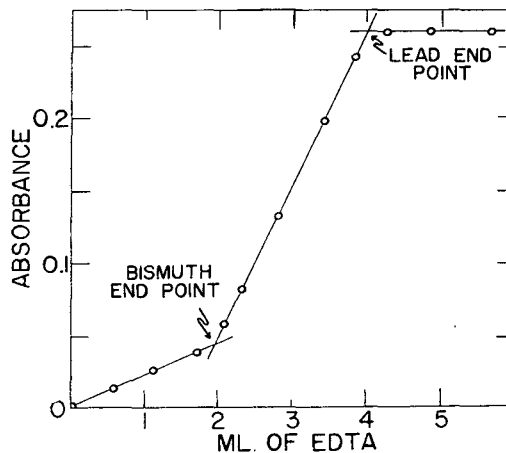(4) Malmstadt, H. V., and Gohrband, E. C., Ibid., 26, 442 (1954).



Figure 3.   Simultaneous titration of bismuth and lead

0.5 mg. of Bi and 0.5 mg. of Pb titrated at 240 mμ

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# Spectrophotometric Determination of Copper with Salicylaldoxime

## Application to Analysis of Aluminum Alloys

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A simple, rapid procedure for the spectrophotometric determination of copper in aluminum and zinc-base alloys has been developed. The method is based upon the formation of copper(II) salicylaldoximate which is extracted into n-amyl acetate from a well buffered aqueous solution of pH about 4.4. At this pH there are no interferences by the elements usually found in aluminum alloys. The copper salicylaldoximate in n-amyl acetate exhibits an absorption maximum at 344 mμ. The range of maximum photometric accuracy is 2.5 to 8.5 × 10⁻⁵ mole per liter in the extracted phase. The method was tested by the analysis of a number of NBS certified standards.

DURING an investigation of the chloroform extraction of some metal salicylaldoximates (5) it was noted that copper salicylaldoximate was extracted quantitatively from aqueous solutions. Because the extracted copper complex was colored and because separation from elements usually found in aluminum alloys was easily effected by control of the pH of the aqueous solution, the method, with slight modifications, was applied to the analysis of aluminum alloys for copper.

When separating cations by the liquid-liquid extraction of metallo-organic complexes, two variables must be controlled: the reagent concentration in the organic solvent, and the hydrogen ion concentration in the aqueous phase. Kolthoff and Sandell

(4) showed that if the formation of the metallo-organic complex in the aqueous phase is given by the equation

$$M^{+n} + nHR = MR_n + nH^+ \qquad (1)$$
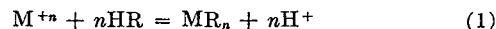
the extractability of the complex into an immiscible solvent can be expressed quantitatively by

$$E = K[HR]_o^n/[H^+]_w^n \qquad (2)$$

where $E$ is the extractability of the metal complex, $K$ is the extractability constant, $[HR]_o$ is the concentration of the reagent in the organic phase, and $[H^+]_w$ is the hydrogen ion concentration in the aqueous phase. The derivation is based upon the mass-action and distribution laws, and the assumptions are made that the reagent and complex are present in both the aqueous and organic phases in the nonassociated forms, and that the metal ion is present only in the aqueous phase. Irving and Williams (3) wrote Equation 2 in the logarithmic form and then differentiated it:

$$(\partial \log E/\partial pH)_{[HR]o} = (\partial \log E/\partial \log [HR]_o)_{pH} \qquad (3)$$

Equation 3 shows directly the effect of changing the reagent and hydrogen ion concentrations: Thus, a change in extractability due to an increase (or decrease) of one pH unit can be exactly offset by a tenfold decrease (or increase) in the concentration of excess reagent in the organic phase. Practical considerations limit the permissible variations in reagent concentration: The upper limit is determined by the solubility of the

organic reagent in the solvent; if the metal complex is to be determined spectrophotometrically, a large excess of reagent would be undesirable if it had an appreciable absorptivity at the wave length used for measurements; and an adequate excess of reagent must be used if sharp separations are to be made, so that the concentration of the reagent does not change appreciably during the extraction. Because of these factors, changes in hydrogen ion concentration are the more important in practical applications of the extraction of metals as complexes, and the reagent concentration should be kept at a constant value.
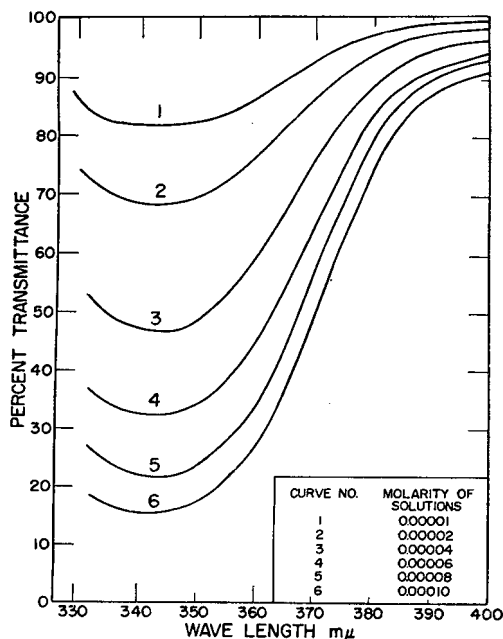


Figure 1.  Absorption spectra of copper(II) salicylaldoximate in *n*-amyl acetate

In this investigation a 0.02$M$ solution of salicylaldoxime in *n*-amyl acetate was used as the extraction reagent. Complete extraction of copper(II) was obtained in the pH range 3.5 to 9.5. The *n*-amyl acetate solution of copper salicylaldoxime exhibited an absorbance maximum at 344 m$\mu$ which was suitable for analytical measurements. The range of maximum photometric accuracy, determined by a Ringbom plot as recommended by Ayres (*1*), was between 2.5 and 8.5 × 10$^{-5}$ mole per liter in the *n*-amyl acetate phase.

### APPARATUS AND REAGENTS

**Instruments.**  Transmittance measurements were made with a Beckman quartz spectrophotometer, Model DU, using a tungsten lamp and blue-sensitive photocell. (Measurements in the range below 330 m$\mu$ were made using a hydrogen discharge lamp.) The instrument was operated at constant sensitivity corresponding to 2.5 turns counterclockwise from the maximum position and with slit widths of 0.60 to 0.70 mm. corresponding to a nominal band width of about 4 m$\mu$.

Matched, stoppered Corex absorption cells with optical paths of 1.000 cm. were used for all measurements above 330 m$\mu$; silica cells were used below 330 m$\mu$.

A Beckman Model H-2 pH meter with a glass-calomel electrode assembly was used to check the pH of all buffer solutions.

**Salicylaldoxime Solution.**  A 0.02$M$ solution was prepared by dissolving 2.7426 grams of salicylaldoxime (Eastman No. 2956) in exactly 1 liter of *n*-amyl acetate.

**Standard Copper(II) Solutions.**  A 0.009997$M$ stock solution of copper(II) was prepared by dissolving 0.6355 gram of electrolytic copper in 6$M$ nitric acid, diluting, boiling to expel oxides of nitrogen, neutralizing the excess acid with 8$M$ sodium hydroxide,

and then making the solution slightly acid with 3$M$ nitric acid. This solution was then diluted to 1 liter. Standards of lower concentrations were prepared as needed by volumetric dilution.

**Buffer Solutions.**  The buffer solutions used to investigate the extraction at various pH values were prepared with the reagents and concentrations as suggested by Clark and Lubs (*2*). The final pH of the buffer solutions was checked with the pH meter.

### PROCEDURE

Extractions were carried out in 30-ml. ground-glass-stoppered bottles, which have certain advantages over separatory funnels: There is no contact of the system with stopcock grease, and mechanical shakers for multiple extractions can be more readily utilized. Ten milliliters of the standard copper(II) solution, suitably buffered to the desired pH, was transferred to the bottle and 10 ml. of the salicylaldoxime solution in *n*-amyl acetate added with a volumetric pipet. Five minutes of shaking was found sufficient for complete extraction (only one extraction is necessary). After the phases had been allowed to separate for 15 minutes, the *n*-amyl acetate phase was transferred, using a medicine dropper or pipet, to an absorption cell for transmittance measurement. If the *n*-amyl acetate phase was transferred immediately, erratic results were obtained due to very small droplets of water still remaining in the organic phase. Best results were obtained by allowing at least 15 minutes for the separation of phases.
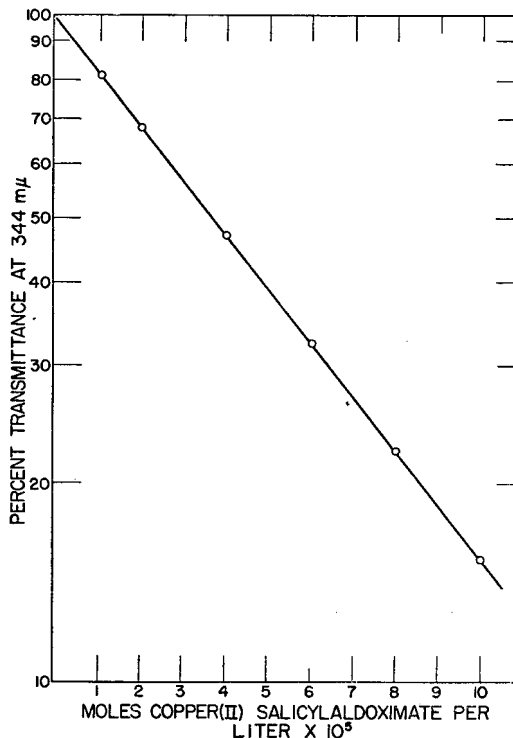


Figure 2.  Calibration curve for determination of copper(II) salicylaldoximate in *n*-amyl acetate

**Absorption and Calibration Curves.**  Standard 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 × 10$^{-5}$$M$ copper(II) solutions were prepared by volumetric dilution of the standard stock solution. The dilutions were made with a buffer solution of pH 4.0. Ten milliliters of the standard solution were extracted with 10 ml. of the reagent solution. A blank was prepared by carrying 10 ml. of the buffer solution through the extraction procedure. The spectral characteristics of the system were evaluated by measuring the transmittance, at frequent wave-length intervals, over the range 320 to 420 m$\mu$. All transmittance measurements were made against the reagent solution which had been carried through the extraction procedure. The absorption curves are shown in Figure 1.

An absorbance maximum suitable for analytical measurements occurred at 344 mμ. A calibration curve was plotted using transmittances measured at 344 mμ (against the reagent solution blank). The color system conforms to Beer's law over the concentration range investigated, as shown by Figure 2.

## RESULTS

**Stability of Color.** Samples extracted from a standard copper solution gave constant transmittance readings over a period of measurement of 48 hours. Several solutions were stoppered and read after a period of several weeks with little change in transmittance.
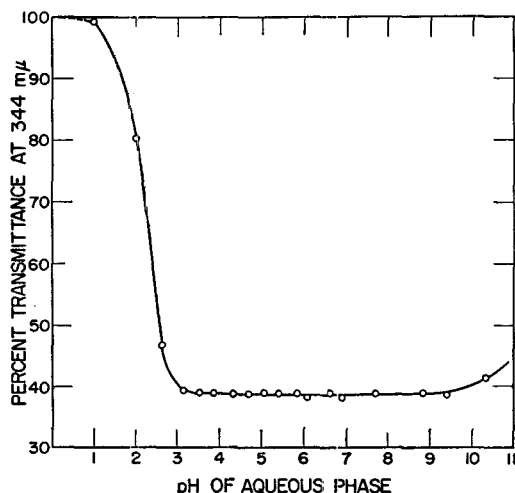


**Figure 3. Effect of pH on extraction of copper(II) salicylaldoximate into n-amyl acetate**
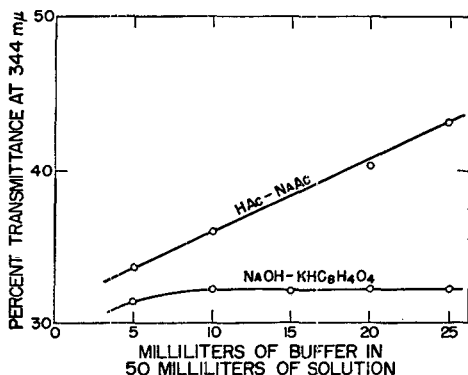


**Figure 4. Effect of buffer concentration on extraction of copper(II) salicylaldoximate into n-amyl acetate**

**Effect of pH on Extraction of Copper Salicylaldoximate.** Standard $5.0 \times 10^{-5}M$ copper(II) solutions were prepared by volumetric dilution of the standard stock solution with buffers of varying pH. Ten milliliters of these standard solutions were extracted with 10 ml. of the reagent solution. The pH study showed that, using $0.02M$ salicylaldoxime in n-amyl acetate, copper(II) could be extracted quantitatively in the pH range 3.5 to 9.5. The results are shown in Figure 3. Above pH 9.5 extraction is not quantitative, apparently because salicylaldoxime acts as a dibasic acid, and copper monosalicylaldoximate is formed in alkaline solutions.

**Effect of Buffer Concentration.** To prevent interference by nickel the extraction was made from solutions of pH 4.4; but the Clark and Lubs buffers did not have sufficient capacity when used with samples that had been dissolved in concentrated acids. A suitable buffer was prepared by adding 75.0 ml. of 0.4M sodium hydroxide to 500 ml. of 0.4M potassium acid phthalate and diluting to 1 liter. The amount of this buffer needed was determined by extracting solutions of pH 4.4 containing a constant concentration of copper(II) with varying amounts of the above buffer solution. The results are given in Figure 4. For each 50 ml. of solution to be extracted, at least 10 ml. of the above buffer must be present to ensure adequate capacity to maintain a constant pH during the extraction. A high capacity acetic acid–sodium acetate buffer of pH 4.0 was also tried with completely unsatisfactory results; the per cent transmittance of the extracted copper salicylaldoximate solution increased linearly with increasing buffer concentration showing no tendency toward leveling off at higher concentrations.

**Table I.   Elements Present in Samples Analyzed**

| Element | Maximum Percentage |
| --- | --- |
| Chromium | 0.24 |
| Iron | 0.90 |
| Magnesium | 1.58 |
| Manganese | 0.81 |
| Nickel | 2.00 |
| Silicon | 0.88 |
| Titanium | 0.10 |
| Zinc | Major constituent in sample 94a |

**Effect of Temperature.** All extractions were made at room temperature, and the spectrophotometer was maintained at 25° C. The transmittance of the copper salicylaldoximate solution in n-amyl acetate showed only the usual temperature coefficient due to the change in volume of the organic solvent with temperature. Because the temperature coefficient of expansion is rather large, it is advisable to make all extractions and transmittance measurements at approximately the same temperature as the calibration.

**Effect of Diverse Ions.** No specific interference studies were made, but the method was applied to the analysis of various aluminum alloys. Copper was satisfactorily determined in the presence of the elements given in Table I by controlling the pH of the aqueous phase. The percentages of the elements given in the table are the maximum values found in all the samples analyzed.

**Table II.   Analysis of Standard Samples**

| NBS Sample | No. of Samples | Copper Found, % Range | Copper Found, % Average | Av. Deviation, % | Certificate Value of Copper, % |
| --- | --- | --- | --- | --- | --- |
| 85a | 16 | 2.48 –2.52 | 2.49 | 0.01 | 2.48 |
| 86c | 12 | 7.80 –7.90 | 7.83 | 0.04 | 7.92 |
| 601 | 12 | 4.30 –4.39 | 4.35 | 0.02 | 4.38 |
| 603 | 5 | 0.274–0.275 | 0.275 | 0.001 | 0.29 |
| 604 | 6 | 3.96 –3.98 | 3.97 | 0.01 | 3.98 |
| 94[a] | 8 | 1.08 –1.09 | 1.08 | 0.01 | 1.08 |

[a] Zinc-base alloy containing 3.90% aluminum.

When silicon is present in large amounts, it is sometimes necessary to remove the silica by filtration prior to extraction in order to get a good separation of phases. Nickel can also be quantitatively extracted, but no interference occurs if the aqueous phase is maintained at a pH below 5.0.

**Application to Samples.** The method was applied to a number of National Bureau of Standards certified aluminum alloys and to one zinc-base alloy. Sample weights of approximately 0 1 gram

were used for analysis of samples containing 2 to 4% copper. Larger sample weights or suitable dilutions were made to adjust other samples to the desired range. The aluminum alloy samples were dissolved by the addition of 5 to 10 ml. of aqua regia, added in small portions to prevent loss of sample. When dissolution was complete, the solution was heated almost to dryness, and then quantitatively transferred to a 250-ml. volumetric flask. The solution was diluted to the mark with distilled water and mixed thoroughly. Twenty-five milliliters of this solution was diluted to 100 ml. with 50 ml. of the buffer solution and distilled water. A 10-ml. aliquot was extracted with 10 ml. of the reagent solution. After waiting 15 minutes for complete separation of phases the n-amyl acetate solution was transferred to an absorption cell and the transmittance measured at 344 m$\mu$ against a blank prepared by carrying 10 ml. of the buffer solution though

the extraction procedure. The results of the analyses are given in Table II.

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ห้องสมุด กรมวิทยาศาสตร์

# Determination of Traces of Nickel in Malt Beverages

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A precise colorimetric method for the determination of traces of nickel in beers and ales is presented. The technique involves ashing the sample, taking up the ash with dilute acid, and shaking out with dithizone in carbon tetrachloride. The soluble, colored nickel complex is formed in the aqueous layer by treatment with dimethylglyoxime, bromine water, and ammonia. The color formed is proportional to the nickel present. Recovery of added nickel is good and copper and iron do not interfere.

A SENSITIVE and precise colorimetric method for the determination of traces of nickel in worts, beers, and brewing materials was developed for investigations on the effect of this metal on fermentation and wildness. This is a continuation in part of the studies conducted in this laboratory on the effects of trace metals in the brewing process.

The presence of traces of nickel in worts and beers is assuming greater importance with the increasing use of nickel or nickel-containing equipment in breweries. The contamination of worts with a few parts per million of nickel may lead to toxic effects on the yeast and slowing of the fermentation. Traces of nickel in the finished beer tend to form gas-evolving nuclei producing the condition of wildness, or gushing beer. This paper presents the details of the technique and data relating to the determination of nickel. The results of tests on the effects of traces of nickel on fermentation and the production of wildness will be presented elsewhere (8).

Hagues (5) in 1931, in a paper on the contamination of beer by copper and nickel, proposed a method which involved dry ashing the sample and finally visually matching the color produced by strongly alkaline dimethylglyoxime with standards in Nessler tubes after standing overnight. The method lacks sensitivity, as 0.6 p.p.m. appears to be the lowest detectable limit. In 1945, Essery (3) noted the many difficulties encountered in the determination of trace metals in wort. After an elaborate dry ashing and solubilization of the ash, he determined nickel by visual comparison of the color produced by oxidation with bromine and treatment with ammonia and dimethylglyoxime. Citric acid was used to prevent interference of phosphates, but it is stated that, if matching is delayed for a short time, precipitation interferes with the color comparison. The author states he obtained a recovery of 93%. The presence of 3 to 4 p.p.m. of

iron produced no interference, but the effect of the presence of traces of copper was not mentioned. After the present method was in use, the paper of Andrews and Harrison (1) appeared in 1954. Their method utilizes a wet digestion to destroy organic matter and the nickel is determined colorimetrically with α-furildioxime in chloroform. The interference of copper is eliminated by extraction of the chloroform solution with dilute sulfuric acid. Very good recoveries are reported. The levels of nickel found in the beers and brewing materials are in line with those found by the authors.

The method reported here is a refinement of the reaction involved in the method of Essery. The reaction was first reported by Feigl (4), who found that nickel and dimethylglyoxime, when oxidized by lead peroxide, produced a red colored compound in alkaline solution. Rollet (6) substituted bromine water as the oxidizing agent and used the reaction to determine nickel quantitatively in steel and various organic compounds. He achieved an accuracy of within 5% and reported that copper interferes. Sandell (7) recommended the separation of nickel from copper by chloroform extraction of the nickelous dimethylglyoxime. Babco (2) investigated the reaction of nickel with dimethylglyoxime in the presence of bromine water and concluded that the mechanism involved oxidation of the dimethylglyoxime and that the order of mixing reagents is important.

The direct determination of nickel in beer, without ashing, was tried, but it is not satisfactory in the range of desired sensitivity. Colored materials are produced from the beer constituents during the test and the colored nickel complex is not readily extractable by solvents from the dark reaction mixture. The method, as here presented, involves dry ashing of the beer or wort. The ash is taken up in dilute hydrochloric acid and extracted with a carbon tetrachloride solution of dithizone to remove the interference of copper and iron. The aqueous layer is treated with dimethylglyoxime and bromine and then made alkaline, developing the brown-to-red nickel color. The intensity of the color, which is directly related to the concentration in the range 0.0 to 0.5 p.p.m., is then read in a photometer. After the ashing step the reactions are conducted in a stoppered 15-ml. graduated centrifuge tube and then transferred to a 10-ml. volumetric flask for color development.

### REAGENTS

Hydrochloric acid, 1 to 1 and 1 to 9.
Dithizone (diphenyl thiocarbazone), 0.05% in carbon tetrachloride. Keep refrigerated in brown bottle.

[1] Present address, The Toni Co., St. Paul, Minn.

Dimethylglyoxime, 1% in 95% ethyl alcohol.

Bromine water, a saturated solution of bromine in distilled water.

Ammonium hydroxide, concentrated (28% ammonia).

Standard nickel solution, nickel ammonium sulfate, $NiSO_4$.-$(NH_4)_2SO_4.6H_2O$, 6.730 grams per liter: 1 ml. = 1 mg. of nickel.

## CALIBRATION OF PHOTOMETER

To graduated 15-ml. centrifuge tubes, add 1 ml. of 1 to 9 hydrochloric acid and aliquots of freshly prepared, appropriately diluted standard nickel solution, keeping the total volume under 6 ml. Cover the range from 0.0 to 0.5 mg. of nickel in suitable increments. Dilute to the 6-ml. mark with distilled water, add 4 ml. of dithizone solution, and continue as described below.

The intensity of color developed is proportional to the concentration of nickel in the above range, so if the response of the colorimeter or photometer employed is linear, a straight-line calibration plot should be obtained and an average calibration factor converting scale readings to parts per million may be calculated. If the response of the photometer is nonlinear, a graphic method with a calibration curve may be used.

## METHOD

Evaporate 100 ml. of wort or "degassed" beer to dryness in a silica dish previously cleaned by boiling with 1 to 1 hydrochloric acid. Char with a Bunsen flame, then ash in a muffle furnace at 500° to 550° C. until ash is white. (In the case of dry materials such as malt, weigh out a suitable charge, such as 10 grams, and char and ash as above.) To the ash add 2 ml. of 1 to 1 hydrochloric acid and 2 ml. of distilled water, taking care to wash down the sides of the dish. Evaporate to dryness on a boiling water bath, and while dish is still hot, take up residue with 1 ml. of 1 to 9 hydrochloric acid and 2 ml. of distilled water. Carefully transfer the acid solution to a graduated 15-ml. centrifuge tube (graduated in 0.1-ml. divisions). Wash out the silica dish with small increments of water, transferring to the tube until the 6-ml. mark is reached.

### Table I. Recovery of Added Nickel

| Sample | Added Metal, P.P.M. | | | Nickel Found, P.P.M. | Added Nickel Recovered, P.P.M. |
|---|---|---|---|---|---|
| | Copper | Iron | Nickel | | |
| Beer A | .. | .. | ... | 0.02 | ... |
| | .. | .. | 0.025 | 0.044 | 0.024 |
| | .. | .. | 0.05 | 0.07 | 0.05 |
| | .. | .. | 0.10 | 0.13 | 0.11 |
| | .. | .. | 0.40 | 0.43 | 0.41 |
| Beer B | .. | .. | ... | 0.01 | ... |
| | 2.0 | 5.0 | ... | 0.01 | ... |
| | .. | .. | 0.025 | 0.031 | 0.021 |
| | .. | .. | 0.05 | 0.05 | 0.04 |
| | 2.0 | 5.0 | 0.05 | 0.06 | 0.05 |
| | .. | .. | 0.10 | 0.12 | 0.11 |
| | 2.0 | 5.0 | 0.10 | 0.12 | 0.11 |
| | .. | .. | 0.40 | 0.41 | 0.40 |
| | 2.0 | 5.0 | 0.40 | 0.41 | 0.40 |

Add 4 ml. of dithizone solution, cork tube tightly, and shake vigorously for 30 seconds, and allow layers to separate. Repeat shaking four times. If color of dithizone turns brownish, draw off spent dithizone and add a fresh 4-ml. portion. Repeat shake-outs and dithizone additions until no change in original dithizone color occurs. Using a 5-ml. volumetric pipet, carefully remove 5 ml. of the aqueous (top) layer and transfer to a 10-ml. volumetric flask. Add 0.5 ml. of dimethylglyoxime solution, mix, and then add 1.0 ml. of saturated bromine water. Mix and let stand 10 minutes. (The solution should be brownish, owing to the excess bromine. If not, add more bromine water.) Then add 0.5 ml. of ammonium hydroxide, mix thoroughly, and diluted to the 10-ml. mark with distilled water. Transfer to a centrifuge tube and centrifuge for 3 minutes at approximately 2500 r.p.m. Read the supernatant liquid (within 15 minutes) in a photoelectric colorimeter, using a green filter and a suitable cell. Set the "zero" reading of the instrument with water. The authors have used a 13-mm. cell and a filter photometer with a composite glass filter having a maximum transmittance at 500 mμ.

A reagent blank is run by mixing 1 ml. of 1 to 9 hydrochloric

acid and 5 ml. of distilled water in a graduated centrifuge tube and proceeding from the step where dithizone is added.

The results are calculated in the usual manner for a colorimetric procedure. If the calibration data are a linear function, multiply the factor (converting photometer readings to parts per million of nickel) by the difference obtained by subtracting the blank photometer reading from the sample photometer reading. If a curvilinear function is obtained for the calibration, pick off the values for the sample and blank from a curve constructed on graph paper relating photometer readings to parts per million of nickel. Subtract the blank results from the sample value to obtain the parts per million of nickel to be reported.

### Table II. Replication of Results

| | Nickel, P.P.M. | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Beer A | 0.01 | 0.01 | 0.01 |
| Beer B | 0.02 | 0.02 | 0.02 |
| Beer C | 0.02 | 0.02 | 0.01 |
| Beer D | 0.04 | 0.04 | 0.04 |
| Beer E | 0.85 | 0.84 | 0.84 |

For beers containing nickel in excess of 0.5 p.p.m., reduce the charge of sample used for ashing. Select a charge such that the color intensity of the solution read in the photometer falls within the value of the calibration curve and gives readings of acceptable precision.

## CALIBRATION DATA

A plot of the calibration data obtained on the Klett-Summerson photoelectric colorimeter gives a straight line, indicating that Beer's law is obeyed in this range of concentrations.

### DATA ON RECOVERY AND INTERFERENCE

To determine the precision and reliability of the method, a series of tests measuring the recovery of added nickel was carried out. Table I gives the results of tests for the determination of the recovery of added nickel in a series of beers. Recovery tests were also conducted on beers to which 2 p.p.m. of copper and 5 p.p.m. of iron were added, to see whether the presence of these levels of copper and iron interfere. Two parts per million of copper and 5 p.p.m. of iron are much above the level of contamination generally found in beer. Inspection of the data shows that excellent recovery of added nickel can be expected from this method and that there is no interference from copper and iron at levels much beyond that ordinarily encountered.

## REPLICATION

Very good replication of results is indicated. Table II contains results of tests conducted in triplicate on a series of beers. Replication is good both at levels of nickel generally encountered from normal beers (beers A to D) and at levels indicating nickel pickup (beer E).

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# Modification of Schwarz von Bergkampf's Method for Determining Aluminum

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Schwarz von Bergkampf's method for determining aluminum in the presence of titanium and iron has been modified to eliminate difficulties with high-iron ferroaluminum alloys when 8-quinolinol is used to precipitate aluminum.

IN THE classical methods for the determination of aluminum in the presence of titanium and iron, it was customary to precipitate the three elements together with ammonium hydroxide and to obtain the sum of the oxides; after determination of iron and titanium, aluminum was obtained by difference. In 1931, Schwarz von Bergkampf (1) proposed a method in which iron was precipitated as the sulfide in the presence of tartrate, after which titanium was precipitated with cupferron without eliminating the excess hydrogen sulfide from the filtrate; he then precipitated aluminum with 8-quinolinol(oxine) in ammoniacal medium without eliminating hydrogen sulfide and excess cupferron.

### Table I.   Determination of Aluminum in Synthetic Samples

| No. | Reducing Agent Added | Al, Mg. Present | Al, Mg. Found |
|---|---|---|---|
| 1 | None | 10.24 | 10.98 |
| 2 | None | 10.24 | a |
| 3 | 50 ml. of saturated H₂S solution | 10.24 | 10.15 |
| 4 | 50 ml. of saturated H₂S solution | 10.24 | 10.22 |
| 5 | 25 ml. of saturated SO₂ solution | 10.24 | 10.24 |
| 6 | 25 ml. of saturated SO₂ solution | 10.24 | 10.23 |
| 7 | 1 ml. of 65% aqueous hydrazine hydrate | 10.24 | 10.20 |
| 8 | 1 ml. of 65% aqueous hydrazine hydrate | 10.24 | 10.20 |

$^a$ Precipitate so contaminated with tarry decomposition products of cupferron that titration was impossible.

This excellent method has been used successfully for many years on materials containing moderate amounts of iron, but when high-iron ferroaluminum alloys containing titanium had to be analyzed, the removal of iron as the sulfide was replaced by electrolysis on a mercury cathode. This change did not affect the cupferron precipitation of titanium in any way, but it caused difficulties in the precipitation of aluminum with 8-quinolinol;

### Table II.   Aluminum Determinations on Iron-Titanium-Aluminum Alloys

| Sample No. | Reducing Agent Used | Al, % Present | Al, % Found |
|---|---|---|---|
| 1 | None | 4.53 | a |
| 1a | 25 ml. of saturated SO₂ solution | | 4.57 |
| 1b | 50 ml. of saturated H₂S solution | | 4.53 |
| 1c | 50 ml. of saturated H₂S solution | | 4.50 |
| 2 | 1 ml. of 65% hydrazine hydrate | 5.91 | 5.87 |
| 2a | 25 ml. of saturated SO₂ solution | | 5.85 |

$^a$ Precipitate contaminated, titration impossible.

when the solutions were made ammoniacal and warmed up the cupferron began to decompose, and the aluminum oxinate was contaminated with tarry decomposition products which made the subsequent titration difficult if not altogether impossible. As this trouble had never been experienced when iron was removed

as the sulfide, it was concluded that the excess hydrogen sulfide remained in solution was protecting the cupferron from oxidation, and this was confirmed experimentally.

It was found that the addition of 25 ml. of saturated hydrogen sulfide or sulfurous anhydride water before making the solution ammoniacal eliminated the trouble completely. In view of the inconvenience in preparing and handling hydrogen sulfide or sulfurous acid solutions, other reducing agents were tried—namely, hydroxylamine hydrochloride and hydrazine hydrate.

### Table III.   Determination of Alumina in High-Iron Bauxites

| Sample No. | Al₂O₃ Found, % Analyst A | Al₂O₃ Found, % Analyst B |
|---|---|---|
| 44 | 56.6, 56.6 | 56.6 |
| 83 | 48.4, 48.4 | 48.8 |
| 125 | 57.3, 57.3 | 57.5 |

These two reagents achieved the intended purpose, but the hydrazine hydrate (or a hydrazine salt) proved to be the most efficient by far. When aluminum oxinate precipitates were digested in the presence of cupferron and hydroxylamine hydrochloride for 0.5 hour, brownish streaks began to appear; similar precipitates digested in the presence of 1 ml. of 65% aqueous hydrazine hydrate (or the equivalent amount of hydrazine sulfate) were still free from cupferron decomposition products after 4 hours.

### PROCEDURE

After removing the iron with a mercury cathode and precipitating titanium with cupferron from an acid solution according to the usual procedure, add to the cupferron-containing filtrate 5 ml. of tartaric acid (100 grams per liter) and 1 ml. of 65% aqueous hydrazine hydrate (or its equivalent in hydrazine sulfate or hydrochloride). A white precipitate of hydrazine sulfate may form but this will disappear when the solution is made ammoniacal and heated. Add rapidly sufficient ammonium hydroxide to provide a 5-ml. excess per 100 ml. of solution, heat to 70° C., and precipitate aluminum with a slight excess of 5% oxine solution in acetic acid. Digest for 30 minutes, filter aluminum oxinate, and complete the determination in the known manner.

### RESULTS

Table I shows results obtained on a pure aluminum sulfate solution of known aluminum content. Ten-milliliter aliquots were treated with tartaric acid, 15 ml. of 36N sulfuric acid, and water to make a 200-ml. total volume. The solutions were cooled to 50° C. and 5 ml. of cold 6% cupferron solution were added, followed by 50 ml. of 1N hydrochloric acid. Such a mixture approximated closely the composition of the filtrate from a cupferron precipitation. Aluminum was next precipitated both in the presence and in the absence of the reducing agents shown in the table. Typical results on iron-titanium-aluminum alloys are shown in Table II. Table III shows results obtained on three high-iron bauxite samples by two analysts working in different laboratories.

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# Determination of Phosphorus in Aluminum and Aluminum Oxide by Radioactivation Analysis

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A method is described for determination of trace amounts of phosphorus in aluminum and aluminum oxide by neutron activation analysis. After a sample is irradiated in a slow neutron reactor, a large, known amount of carrier phosphorus is added, then a conventional chemical separation for phosphorus is made. The radioactivity of the separated phosphorus is compared with that of a standard treated in the same manner to give the phosphorus content of the unknown. Phosphorus contents in the range of 0.001 to 0.0001% were determined to 5% of the absolute value. A practical sensitivity of 0.0000005% phosphorus is possible.

PRACTICALLY all methods for the separation of phosphorus are based on precipitation of ammonium phosphomolybdate. Numerous procedures for the subsequent treatment of this precipitate have been proposed; it may be redissolved and precipitated as magnesium pyrophosphate or the phosphorus may be estimated directly from measurement of the yellow precipitate by gravimetric, volumetric, or colorimetric means.

The determination as magnesium pyrophosphate is not applicable in the case of commercial aluminum alloys and refined aluminum oxides where the phosphorus contents are very low, and the sample size is limited by solution difficulties. The other procedures depend upon a precipitate of the formula $(NH_4)_3PO_4$. $12MoO_3$, which, however, generally contains slightly more molybdic oxide than is indicated (2). Also, small amounts of silica, always encountered in the oxide or flux mixture, cause an increase in the mole ratio of molybdenum to phosphorus (1). The presence of silica precludes the use of a colorimetric method, since silicon forms a compound with molybdenum that is reduced to a blue color. Also, the presence of aluminum interferes in the molybdenum blue reaction (3).

The technique of radioactivation analysis circumvents many of the difficulties usually encountered in the determination of trace elements. There are a number of descriptions of this technique in the recent literature (5-7).

The technique can be simply stated. When any material is irradiated in a chain-reacting, neutron reactor, the activity induced in any given element can be calculated from the expression:

$$A(t) = (1.63 f\sigma\phi \times 10^{13})(1 - e^{-\lambda t})$$

where

$A(t)$ = activity per gram atom
$f$ = isotopic abundance of the parent element
$\sigma$ = cross section in barns
$\phi$ = neutron flux in neutrons/sq. cm. sec.
$\lambda$ = decay constant
$t$ = time of irradiation

The reliability of the parameters in this relation is generally well known with the exception of the neutron flux, $\phi$. This is uncertain, and without detailed knowledge of the operation of the pile throughout the period of irradiation there is no assurance of its magnitude or constancy.

The usual way to circumvent this difficulty is to irradiate along with the unknown, a small quantity of a pure compound of the element to serve as a standard. After irradiation, a known quantity of carrier of the element is added and a conventional chemical separation is carried out in both the standard and the unknown. The activities of the separated material are then compared under identical counting conditions. The ultimate sensitivity is determined by the degree to which the element can be separated radiochemically pure; or, if impure, by the degree to which the desired activity can be measured in the presence of the interfering activities.

Phosphorus lends itself particularly well to determination by radioactivation analysis. It has a fair activation cross section of 0.23 barn. Its intermediate half life of 14.3 days is long enough to permit the decay of many possible interfering activities, but short enough to permit the ascertainment of radiochemical purity by half-life measurement. Moreover, it is a pure beta emitter and can be readily distinguished by absorption measurements from most contaminants that have associated gamma emission.

## EXPERIMENTAL

**Activation.** For irradiation, the samples were sealed in the customary Oak Ridge–type of container. This consists of a commercial purity (1100 alloy) aluminum, impact-extruded can, $7/_8$ inch in diameter by $3^3/_8$ inches long. The aluminum alloy samples were in the form of turnings or strips of thin sheet. The aluminum oxide samples and the standards were sealed in individual capsules made by closing the ends of thin-walled, 1100-aluminum tubing $1/_4$ inch in diameter.

### Table I. Attenuation of Neutron Flux in Standard

| Weight of $NH_4H_2PO_4$, Gram | Relative Specific Activity |
|---|---|
| 0.0012 | 1.00 ± 0.05 weighing error |
| 0.0136 | 1.03 ± 0.01 probable counting error |
| 0.0586 | 1.03 ± 0.01 probable counting error |

In some cases several irradiation cans were required to contain all of the samples of one series. In these cases, a separate standard was contained in each can.

The samples were activated for one week in the thermal neutron reactor operated by the Union Carbide and Carbon Corp. at Oak Ridge, Tenn. They were placed in the 14-Z positions of the pile. These positions are in the outer "cool" part of the pile where the fast neutron population is very low.

**Attenuation Effects in Standard.** An initial study was made to determine the most suitable phosphorus standard to accompany the samples. In the general case, a compound made up of elements whose absorption for neutrons is low in comparison to the standard element is desirable to minimize self-attenuation effects. The ammonium phosphates aptly fulfill this requirement, as nitrogen, hydrogen, and oxygen do not have large cross sections. Diammonium monohydrogen phosphate decomposed slightly at 130° C. overnight and was not suitable for that reason. Monoammonium dihydrogen phosphate was stable and was used as the standard.

If self-attenuation occurs, it is made apparent when the specific activities of samples of considerably different size are

compared, and the effect can be corrected for by extrapolating the activity of a range of sample sizes down to zero weight.

In a separate experiment, approximately 0.001-, 0.01-, and 0.05-gram samples of the standard were irradiated and the relative specific activities were determined by counting equal-weight portions of their ammonium phosphomolybdate precipitates. The results are given in Table I.

It is seen that self-attenuation effects in the standard were absent. For the subsequent activations, a standard of approximately 0.02 gram was employed as a conveniently weighable amount.

**Attenuation in Samples.** Errors could also result if the samples were sufficiently opaque to neutrons to result in an appreciable attenuation of the flux throughout the thickness of the material. The comparison of the measured activity with that of the standard would then no longer be valid, as the activity would not be strictly proportional to the content of the element in question. In the case of the metal samples, the composition was sufficiently well known from the analysis and history of the material so that there could be assurance that self-attenuation would be absent. Such information was not as complete in the case of the aluminum oxides, however. Boron was the most likely high cross section contaminant in the oxides, but its concentration was established spectroscopically to be below 0.01%. No information was available, however, on the concentration of the rare earth elements and, since some of these have extremely high cross sections, very minute amounts could be deleterious.

A supplementary experiment was carried out with one representative oxide. Approximately 0.1-, 0.5-, and 1 gram samples of this material were irradiated, and the relative specific activities of their phosphomolybdate precipitates were compared. The results are shown in Table II.

**Table II. Attenuation of Neutron Flux in Sample**

| Weight of Sample, Gram | Relative Specific Activity |
|---|---|
| 0.1 | 1.00 ± 0.01 probable counting error |
| 0.5 | 0.98 ± 0.01 probable counting error |
| 1.0 | 0.98 ± 0.01 probable counting error |

Self-attenuation effects are seen to be absent within the error of the measurement and, since the chemical history of the oxide samples was similar in all cases, this result would be applicable to the others.

**Variation of Flux over Cans.** Since the thermal flux distribution in the pile extremities was unknown, it was necessary, by determining the maximum variation of the flux over the dimension of a can, to establish the maximum error that could result if the flux over the standard were different from that over the sample.

From the construction of the pile, flux gradients of two sorts would be expected. Since the graphite stringers that contain the cans are inserted at right angles between the fuel rods, a variation in flux over the length of the can might be expected. Also, since the total flux falls off rapidly toward the edge of the structure, a variation from one can to another, or across the diameter of a single can, might be expected.

The flux distribution over the length of the can was established by employing a neutron-monitoring foil as a liner in each irradiation can. This consisted of 0.003-inch-thick, 3003 aluminum foil that contained about 1% manganese. The flux distribution was determined by measuring the activity of small, weighed squares cut from the foil at the top, center, and bottom. Throughout the investigation there was a very slight indication that the neutron flux at the top and the bottom of the can was greater than that in the center. The maximum spread in any

instance was only 4.9%, and this was taken into account in evaluation of the final results.

Substantial differences were found, however, between the average activity of the foils in different irradiation cans. In one series, the samples were contained in four different cans located in the graphite stringer in positions 14-Z-19 to 22. The relative activities induced in the foils in these four positions were 1.00, 1.07, 1.20, and 1.24. A 24% difference in flux between the outermost and the innermost cans in the stringer was seen. Since these two cans (19 and 22) were separated by approximately 8 inches, a flux gradient of approximately 3% per inch existed. This introduces a possible maximum error of 2.5% over the diameter of the can. This maximum error could only be realized, however, if the standard were entirely against one wall of the can and the sample were entirely against the wall diametrically opposite in the direction of increasing or decreasing flux.

A similar exploration was made in other series where more than one irradiation can was employed. A similar flux distribution was observed, so a 2% possible error from this variable was considered reasonable throughout the investigation.

**Counting Procedure.** The counting planchet consisted of an aluminum disk 1 inch in diameter by $1/4$ inch thick with a conical-bottom hole $3/8$ inch in diameter by $3/16$ inch deep drilled in the center of the top face. The conical bottom of the hole served to center small precipitates, whereas large precipitates could be packed uniformly over the entire $3/8$-inch diameter.

The samples were contained in a 2-inch-thick lead shield for counting. The Geiger tube was a halogen-filled, end-window type obtained from the Nuclear Instrument and Chemical Corp.

A 45 mg. per sq. cm. aluminum filter was generally interposed between the sample and tube to minimize the error from small variations in sample thickness. The filter was not used, however, with samples that contained less than 0.001% phosphorus because of the low counting rate.

A total of 10,000 counts or greater was taken on all samples, with the exception of the very low phosphorus alloys where 3000 counts or greater was taken. Counts were made over a 1- to 2-week period, and values were taken from the best 14.3-day line through the points.

**Chemical Procedure.** For one series of aluminum oxides, 1 gram of activated sample, 0.013 gram of nonradioactive monoammonium dihydrogen phosphate carrier, and 6 grams of borax-carbonate flux mixture were added to a platinum crucible. The mixture was heated for at least 30 minutes at the highest temperature of a Meker blast burner, then the fusion was cooled and dissolved in dilute nitric acid. A standard was prepared by fusing a like mixture containing 1 gram of inert alumina instead of the activated sample. To the solution of this melt was added a known amount of the radioactive monoammonium dihydrogen phosphate standard.

The acidity of the solutions was adjusted, and all the phosphorus was oxidized to phosphate with potassium permanganate. The phosphorus was precipitated as phosphomolybdate, using ammonium molybdate-citrate solution. This precipitate was dried and weighed, then 0.1 gram was transferred to a planchet and counted. After counting, the precipitate was dissolved and the phosphorus precipitated as magnesium ammonium phosphate, which was ignited to magnesium pyrophosphate and counted in that form.

For the remainder of the aluminum oxides, the phosphomolybdate precipitate was dissolved without drying and reprecipitated as a purification step.

For the aluminum alloys, 2 grams of the metal, 0.25 gram of mercurous nitrate, and 0.013 gram of the carrier were dissolved in 100 ml. of 1 to 1 nitric acid. After removal of most of the acid by boiling, the solutions were gassed with hydrogen sulfide, filtered, and boiled. After adjusting the acidity, part were doubly precipitated as phosphomolybdate and the remainder only singly precipitated.

The several procedures above were employed with different series of samples to see which variation gave precipitates most radiochemically pure. The purity was established by decay rate determinations taken over one to two half lives. All precipitates, regardless of the procedure employed, appeared to be pure, with the exception of the phosphomolybdates from one

aluminum oxide series. These contained traces of tungsten as demonstrated by analysis of the composite decay curves. The 24.1-hour component of tungsten-187 was readily distinguished and an accurate difference curve could be plotted. The 73.2-day component of tungsten-185 was not perceptible so was disregarded.

The tungsten contamination was not surprising, since tungsten coprecipitates with phosphorus as the insoluble ammonium phosphotungstate.

In comparing the specific activity of an unknown precipitate with that of a standard, it is imperative that the counting geometry be identical. It is particularly necessary in the case of a pure beta emitter that the thickness (on a weight basis) of the unknown and standard be identical. This requirement was more nearly met with the phosphomolybdate precipitate than the phosphate. The phosphomolybdate was very voluminous. It powdered readily and could be compacted firmly into the counting planchet by gentle shaking. The pyrophosphate, on the other hand, was very dense and refractory after ignition, and was difficult to spread uniformly over the counting planchet.

The double precipitation as the ammonium phosphomolybdate evolved as the preferred procedure with the reservation that tungsten, if present, would follow the phosphorus, but could be corrected for with a high degree of accuracy.

### RESULTS AND DISCUSSION

Some representative phosphorus determinations are shown in Table III. These are given to illustrate the precision and reproducibility of the method, and should not be taken as necessarily typical of the particular materials. The sets of values are duplicate or triplicate determinations of the same sample with the exception of the values for 1100 sheet that were from two different lots of alloy.

#### Table III. Phosphorus Determinations by Radioactivation Analysis

| Material | Description | % P | Material | Description | % P |
|---|---|---|---|---|---|
| Al$_2$O$_3$ | Alcoa | | | | |
| | 1 | 0.00020<br>0.00021 | Al | 1100 extrusion | 0.000053<br>0.000056 |
| | 2 | 0.00013<br>0.00013 | | 1100 sheet | 0.000059<br>0.000067 |
| | 3 | 0.00023<br>0.00023 | | High-phos-<br>phorus pig | 0.0029<br>0.0029<br>0.0027 |
| | French | | | | |
| | 1 | 0.00051<br>0.00053 | | High-phos-<br>phorus sheet | |
| | 2 | 0.0018<br>0.0017 | | 1 | 0.0013<br>0.0013 |
| | 3 | 0.0012<br>0.0013 | | 2 | 0.0019<br>0.0019 |
| | Refined phos-<br>phate rock | 0.0034<br>0.0034<br>0.0035 | | 3 | 0.0019<br>0.0021 |

A variable whose magnitude was not determined experimentally was the production of radioactive phosphorus from sulfur by the $(n,p)$ reaction. This is a common pile reaction for producing carrier-free phosphorus.

The sulfur content of the alloy samples was negligible. In the oxide samples, however, sulfur contamination was possible, and particularly in the material that was refined from phosphate rock by a sulfate process the sulfur content might have been appreciable.

It is known that the reaction probability of the sulfur-32 $(n,p)$ phosphorus-32 reaction with low energy neutrons is extremely low. Klema and Hanson (4) found that the cross section leveled off at about 0.3 barn at about 4 m.e.v. It fell off very rapidly at lower neutron energies. The threshold energy

was about 1 m.e.v. The neutron population of this energy in the extremities of the pile where the samples were irradiated is low, so this possible source of error was disregarded.

Since a large, constant amount of carrier was added to each sample, weighing errors were constant regardless of the phosphorus content. The smallest quantity that was weighed was the 0.01 gram of carrier added to each sample, so weighing errors were considered negligible.

The maximum error from variation of the neutron flux over the dimensions of the can was 2%.

A possible error was the contamination of the precipitates by other radioactive elements with radiological properties similar to those of phosphorus. The only such isotopes with half lives between 12 and 17 days are barium-140, cesium-136, osmium-191, vanadium-48, palladium-103, and tellurium-121. Only osmium need be considered as a possible contaminant. The remainder are excluded either because they do not result from neutron-gamma reactions, or have small cross sections and are produced from isotopes of very low abundance. Osmium, with a 15.0-day half life, could be present to an appreciable extent and not be distinguished by half-life determinations alone. Its cross section of 0.6 barn would give about threefold higher yield than phosphorus if both were present to the same extent. This would require, however, that osmium follow phosphorus exactly through the chemical separation. Though the details of osmium chemistry are not well known, this latter is unlikely, since osmium tetroxide would probably be lost from the boiling nitric acid solutions. Moreover, osmium has a very weak beta (0.14 m.e.v. compared to 1.7 for phosphorus) and has associated gamma emission. Such radiation would be detected by absorption measurements, though admittedly this technique is not sufficiently sensitive to detect traces of such contaminants. Considering also that osmium is a very unlikely contaminant in the materials under study, its contribution has been disregarded.

The largest error in the results of the low-phosphorus samples undoubtedly was a statistical error because of the low counting rate. This appeared to be about 5% in the worst case.

Everything considered, it appears that the maximum probable error in the determinations was about 10% in the low-phosphorus samples and possibly 5% in the high-phosphorus samples.

Some consideration has been given to the ultimate practical sensitivity of the method with the materials described here. As much as 10 grams of sample could be handled without difficulty to give another order of magnitude in counting rate. Irradiation times of 2 half lives would increase the activity by about 50%. Counting rates of about 25 counts per minute above background could be measured to perhaps 3% probable error if measurements were repeated over several days. This would give a "practical" sensitivity with the counting equipment described of about 0.0000005% phosphorus, determined with an error of about 10%, assuming no impurities came in at this concentration that could not be recognized and corrected for.

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# Saponification Method for Rosin Esters

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The saponification time of rosin esters is reduced by the use of hydrazine. Dark grades of rosin are bleached by the hydrazine, so that a phenolphthalein end point is easily seen. Good reproducibility and agreement with theoretical values were obtained using a saponification time of 1 hour.

THE increased use of complex esters of rosin has made it necessary to develop a simple and accurate saponification method suitable for routine use. Most procedures for the saponification of rosin esters (1-5) require special care to avoid formation of dark solutions that are difficult to titrate. Some saponification solutions are viscous and difficult to pipet accurately.

The method described involves the use of a small amount of hydrazine in the solution used for saponification. Its presence aids in the saponification and it also acts as a decolorizing agent for many of the color bodies commonly found in rosin esters.

## REAGENTS

**Hydrochloric Acid Solution (0.25 to 0.50N)**, accurately standardized.

**n-Hexyl Alcohol–Potassium Hydroxide Solution.** Dissolve 40 grams of reagent grade potassium hydroxide and 20 ml. of 85% hydrazine hydrate in 1 liter of n-hexyl alcohol. Allow this solution to stand 24 hours and then filter to remove any insoluble matter. The n-hexyl alcohol used to prepare this solution should be redistilled from potassium hydroxide, over which it has been standing overnight, or with which it has been refluxed for 1 hour.

**Phenolphthalein Solution.** Dissolve 1 gram of phenolphthalein in 100 ml. of 95% alcohol, and neutralize the slightly acid alcoholic solution with sodium hydroxide solution.

## PROCEDURE

Weigh 1.00 ± 0.001 gram of the sample of rosin ester into a 250-ml. alkali-resistant saponification flask. Pipet 25 ml. of the hexyl alcohol–potassium hydroxide solution, allowing the pipet to drain for a definite time. Add a few particles of silicon carbide boiling promoters. Connect the flask preferably to a water condenser or to an air condenser made from glass tubing, 15-mm. inside diameter, having a minimum length of 32 inches.

Place on a hot plate and boil the solution gently for at least 1 hour. Remove the condenser and cool to room temperature.
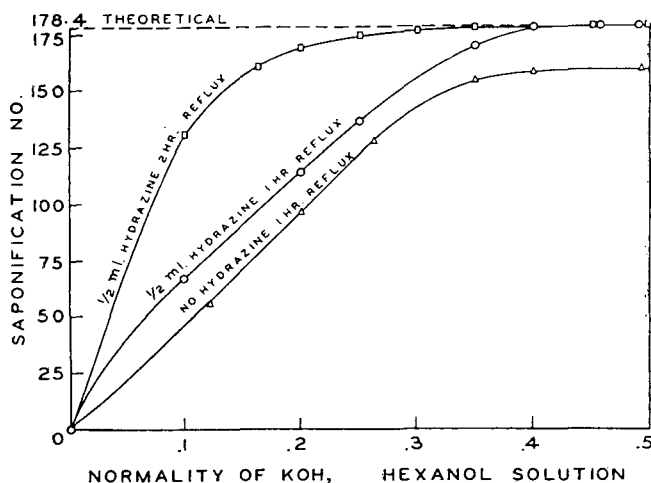


**Figure 1. Effect of hydrazine on alkali saponification of rosin esters**

1.000 gram of methyl dehydroabietate in 25 ml. of reagent

## Table I. Saponification of Rosin Esters[a]

| | Sap. No. |
|---|---|
| Methyl dehydroabietate, m.p. 62.5– 63.5° C. (6), theor. sap. No. = 178.4 | 177.8 |
| | 178.4 |
| | 179.0 |
| | 178.0 |
| Av. | 178.3 |
| Lactone of hydroxytetrahydroabietic acid, m.p. 130.5–131.5° C. (7), theor. sap. No. = 184.6 | 184.9 |
| | 184.3 |
| | 184.9 |
| | 184.5 |
| Av. | 184.5 |
| WW gum rosin | 181.1 |
| | 180.8 |
| | 181.0 |
| | 181.3 |
| Av. | 181.0 |
| N wood rosin | 173.1 |
| | 173.4 |
| Glycerol ester of rosin | 168.8 |
| | 168.0 |
| Pentaerythritol ester of gum rosin | 149.0 |
| | 150.0 |
| Maleic modified glycerol ester of rosin | 233.2 |
| | 233.4 |
| Phenol formaldehyde–modified glycerol ester of rosin | 132.9 |
| | 133.2 |

[a] 1.000-gram sample, 25 ml. of 0.5N potassium hydroxide solution in n-hexyl alcohol containing 20 ml. of 85% hydrazine hydrate per liter, refluxed gently for 1 hour.

Add 50 ml. of neutral ethyl alcohol, or reagent grade isopropy alcohol, and titrate with the standard hydrochloric acid solution, using 1 ml. of the phenolphthalein solution.

Conduct a blank determination on 25 ml. of the hexyl alcohol–potassium hydroxide solution, using the same pipet and draining for the same length of time.

## RESULTS

When saponified for 1 hour by the foregoing procedure, purified methyl dehydroabietate, melting point 62.5–63.5° C. (6), was found to have a saponification number of 178.3. Theoretical value is 178.4. This value was duplicated within ±0.7 unit.

Upon acidification of the saponified solution of this ester, the dehydroabietic acid precipitated readily and after one crystallization from hot ethyl alcohol it had a melting point of 167° to 170° C. (6).

Samples of the lactone of hydroxytetrahydroabietic acid, melting point 130.5–131.5° C. (7), gave saponification numbers of 184.9 to 184.3, after refluxing for 1 hour. Theoretical for this lactone is 184.6. The hydroxytetrahydrobietic acid which precipitated upon acidifying the saponification solution had a melting point of 155° to 160° C. (7). The results obtained with several rosin esters are listed in Table I.

## DISCUSSION

The hydrazine–hexyl alcohol saponification method possesses several advantages.

The hexyl alcohol solution of potassium hydroxide and hydrazine is water white and remains so after continuous reflux. The solution can be accurately pipetted. A blank on the reagent had not changed after standing for 12 weeks. A period of 1-hour reflux was sufficient for all esters tested, and in most cases the saponification is complete within 30 minutes. Dark samples such as FF wood rosin are bleached by the hydrazine to the extent that a phenolphthalein end point is easily seen; accuracy and precision are easily obtained.

The alcoholic potassium hydroxide–hydrazine reagent was prepared using all of the straight-chain primary alcohols below hexyl.

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The results of saponifications using these alcohols indicated that such reagents were impractical, inasmuch as increased basicity developed which was titrated at the phenolphthalein end point. However, the hydrazine–potassium hydroxide solution diluted with butyl alcohol or amyl alcohol gave satisfactory results when the test solution was carefully evaporated to dryness at the end of the saponification, then redissolved in alcohol, and titrated. Methyl dehydroabietate saponified by this method gave a saponification number 177 (theoretical 178.4). With all alcohols below n-amyl, gas was evolved and the blank on the reagent gradually increased on standing.

Figure 1 shows the merits of using hydrazine in alkali saponification of rosin esters. When solutions less than 0.4N are used a reflux period of 2 hours is required to complete the reaction.

However, 1 hour is sufficient with a 0.4 to 0.5N potassium hydroxide solution.

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# Determination of Cyanide, Thiocyanate, and Alpha-Hydroxynitriles in Plasma or Serum

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A novel technique is described for the estimation of cyanide, thiocyanate, and α-hydroxynitriles in serum or plasma. It is based on the conversion of cyanide and thiocyanate into cyanogen bromide which is subsequently reacted with benzidine in pyridine to give an intense color.

IN A study of methods for the determination of cyanide and thiocyanate ions in biological material it was found that a modification in the technique of the Aldridge (2) method would enable the analyst to determine the concentration of cyanide and thiocyanate in serum or plasma separately, rather than calculate the concentration of cyanide by difference as is done by Aldridge. This results in a greater degree of accuracy, especially in the analysis of samples containing trace quantities of cyanide in the presence of large quantities of thiocyanate.

## APPARATUS

A Beckman Model DU spectrophotometer was used with 1.000-cm. cuvettes for light measurements.

An aeration apparatus consisting of three test tubes connected in series is shown in Figure 1. Test tubes A (containing 25 ml. of 20% sodium hydroxide) and B are 1 × 8 inches and are fitted with aeration bulbs. Test tube C is 20 × 150 mm. and is fitted with the inlet tube drawn to a capillary tip. Air entering at·E is drawn from a source outside of the laboratory. Vacuum pumps attached to D.

## REAGENTS

**Arsenous Acid.** A 2.0% solution of arsenous acid is prepared by refluxing 2.0 grams of arsenous acid with distilled water until solution is complete. It is then diluted to 100 ml.

**Bromine water.** A saturated solution of bromine in distilled water is used.

**Benzidine Hydrochloride Solution.** A 4.0% solution of benzidine hydrochloride in water is prepared freshly each day.

**Pyridine Solution.** This solution is prepared by adding 100 ml. of concentrated hydrochloric acid to 1 liter of 60% pyridine in water (v./v.).

**Trichloroacetic Acid.** A solution of 20% trichloroacetic acid (w./v.) in distilled water is prepared.

**Stock Standard Cyanide Solution.** A solution containing approximately 50 mg. of sodium cyanide in 100 ml. of 1.0N sodium hydroxide is prepared. The exact concentration is determined by titrating with 0.02N silver nitrate using 20% potassium iodide as indicator. From this stock solution, a series of working solutions is prepared by dilution to contain from 0.05 to 2.0 γ of cyanide per 1.0 ml. in 1.0N sodium hydroxide for preparation of the calibration curve. As cyanide solutions are very unstable, this solution should be standardized and diluted immediately before it is used.

**Stock Standard Thiocyanate Solution.** A 0.02N solution of ammonium thiocyanate is prepared and standardized against 0.02N silver nitrate, using iron(III) alum as indicator. From this stock solution, a series of working solutions is prepared by dilution to contain from 0.10 to 2.3 γ of thiocyanate per 1.0 ml. for preparation of the calibration curves.

**Pyridine-Benzidine Solution.** Immediately before use, 1 part by volume of benzidine hydrochloride solution is mixed with 5 parts by volume of the pyridine solution.

## PRELIMINARY PROCEDURE

**Wave Length Selection.** Absorption curves for the colors developed for $2.3 \times 10^{-5}M$ solutions of cyanide and thiocyanate are shown in Figure 2. These curves have the same shape and indicate that the same material is being measured in each case. A maximum in absorbance is present in each at 532 mμ. This wave length was therefore selected for maximum sensitivity.

**Calibration Curve.** Calibration curves were prepared for cyanide and thiocyanate by carrying aliquots containing known amounts through the color development procedure exactly as described. The slope of the curve for thiocyanate is 0.281 and
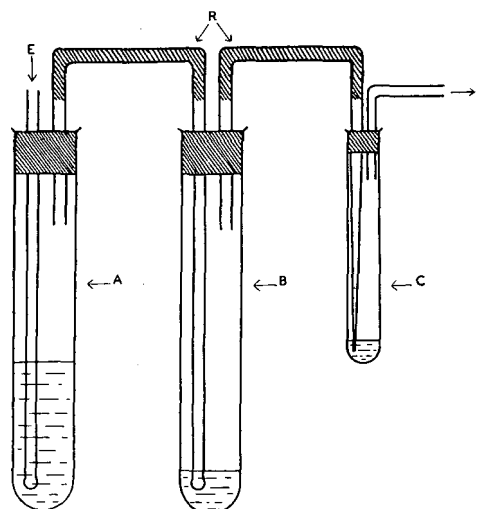


**Figure 1.  Aeration apparatus**

A.   Air washing tube
B.   Aeration tube to contain sample
C.   Receiving tube
D.   To vacuum
E.   Air inlet
R.   Rubber tubing connections

that for cyanide is 0.645. The ratio of these is 2.30; the reciprocal ratio for molecular weights of cyanide to thiocyanate is 2.23, showing very good agreement.

### ANALYTICAL PROCEDURE

**Determination of Cyanide and Thiocyanate in Plasma.** Exactly 5.0 ml. of 20% trichloroacetic acid is placed in tube B, and 0.50 ml. of 0.10N sodium hydroxide in the receiving tube. The test tubes are connected with the rubber tubing and the receiving tube is stoppered tightly. The sample, usually 1.0 ml., is pipetted into tube B, which is stoppered immediately, and aeration is begun. Aeration is continued for 15 minutes at a rapid rate.

At the end of this time the apparatus is disconnected. The contents of the receiving tube are then analyzed for cyanide. The contents of the capillary inlet tubing of the receiving tube are blown out and then washed out into the receiving tube with 0.50 ml. of 20% trichloroacetic acid. One drop of saturated bromine water is added immediately and the contents are mixed. To remove the excess bromine, 0.20 ml. of the arsenous acid solution is added and again mixed. The vapors of bromine above the solution are blown off with a stream of air; 3.6 ml. of pyridine-benzidine mixture is then added and color is allowed to develop for 15 minutes. The absorbance of the red color is then measured in the spectrophotometer at 532 mμ. The concentration is determined from a previously prepared calibration curve.

The thiocyanate is determined in the residual mixture in tube B after aeration of the cyanide. The mixture in tube B is filtered through Whatman No. 42 filter paper; 1.0 ml. of this protein-free filtrate is transferred to a 20 × 150 mm. test tube. The procedure then followed is exactly the same as that used for cyanide determination beginning with the addition of 1 drop of bromine water. A dilution of 1 tb 6 of plasma is used in this determination if the above procedure has been followed. Calculation of the concentration of thiocyanate from a calibration curve must take this into consideration.

**Determination of α-Hydroxynitriles.** α-Hydroxynitriles under alkaline conditions liberate hydrocyanic acid. This is used as a basis for the analytical procedure for the determination of lactonitrile and glycolonitrile in plasma or serum.

In the presence of free cyanide and thiocyanate, the cyanide is determined in the manner described above. After the cyanide

is removed by aeration, the receiving tube is replaced by another, also containing 0.5 ml. of 0.1N sodium hydroxide. Then 2.0 ml. of 10% sodium hydroxide is added to the tube, containing the sample in trichloroacetic acid, to make the mixture alkaline. The tube is allowed to stand for 5 minutes to hydrolyze the α-hydroxynitrile. (Hydrolysis for periods up to 15 minutes at 60° C. did not give significantly different results from those described.) The mixture is then made acid again by the addition of 7.0 ml. of 20% trichloroacetic acid and aeration begun immediately. After 15 minutes of aeration, the receiving tube is removed and the colorimetric procedure carried out for cyanide. The concentration of nitrile present in the plasma is calculated from the cyanide found. Thiocyanate in the sample is determined as previously described. The dilution of thiocyanate in this case is 1 to 15.

**Interference.** Aldridge (1) has discussed the effect of temperature, hemolysis, and the interference of various compounds. Any compound that will react to form cyanogen bromide under the conditions of the analysis will interfere; however, these are not normally present in blood.

### RESULTS

Table I shows the results obtained when known amounts of cyanide and thiocyanate were added to plasma. Table II presents the recovery of hydrolyzed cyanide from known amounts of two α-hydroxynitriles—glycolonitrile and lactonitrile—from aqueous solutions.
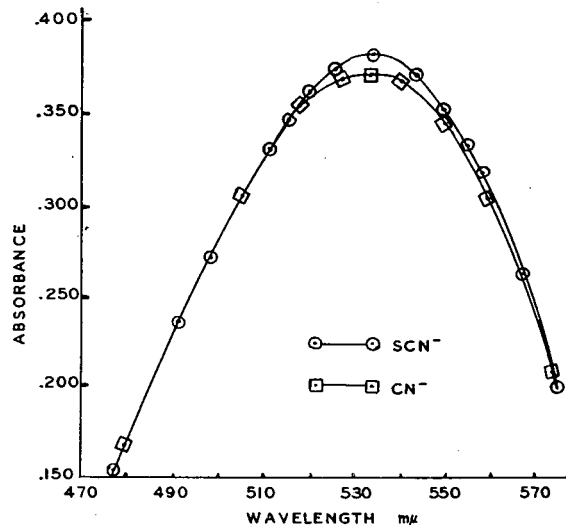


**Figure 2. Absorption curves of color developed by 1.0 ml. of 2.3 × 10⁻⁵M solutions of cyanide and thiocyanate**

Aliquots of solutions containing varying amounts of the nitriles were treated with 0.1N sodium hydroxide to liberate the cyanide. The solutions were then made acid with trichloroacetic acid and carried though the procedure for cyanide. The average recovery of hydrolyzed cyanide was 89.4 and 91.0% from aqueous solutions of glycolonitrile and lactonitrile, respectively. As the average extent of hydrolysis was about 90% for the nitriles, the calculations should be made from calibration curves prepared by carrying the individual nitrile through the procedure.

Data on the recovery of lactonitrile added to plasma in the presence of added cyanide and thiocyanate are presented in Table III. The amount of each was present in varying proportions and satisfactory recoveries were obtained.

#### Table I. Recovery of Cyanide and Thiocyanate from Plasma

| Cyanide | | | Thiocyanate | | |
|---|---|---|---|---|---|
| Added, γ | Found, γ | Recovery, % | Added, γ | Found, γ | Recovery, % |
| 0.03 | 0.03 | 100 | 0 | 1.56ᵃ | |
| 0.08 | 0.08 | 100 | 0.58 | 2.14 | 100 |
| 0.22 | 0.21 | 96 | 1.16 | 2.70 | 98 |
| 0.60 | 0.57 | 95 | 1.16 | 2.74 | 102 |
| 0.88 | 0.93 | 106 | | | |
| 1.20 | 1.24 | 103 | 0 | 1.61ᵃ | |
| 1.76 | 1.63 | 93 | 0.88 | 2.47 | 98 |
| 2.00 | 2.02 | 101 | | | |
| Average | | 99 | | | 100 |

ᵃ Sample of plasma originally contained this amount of thiocyanate.

#### Table II. Recovery of Cyanide from Glycolonitrile and Lactonitrile

| Glycolonitrile | | | Lactonitrile | | |
|---|---|---|---|---|---|
| Cyanide, γ | | Recovery, % | Cyanide, γ | | Recovery, % |
| Added | Found | | Added | Found | |
| 0.26 | 0.25 | 96 | 0.18 | 0.16 | 89 |
| 0.53 | 0.47 | 89 | 0.35 | 0.32 | 91 |
| 0.79 | 0.70 | 89 | 0.53 | 0.48 | 91 |
| 1.05 | 0.92 | 88 | 0.70 | 0.64 | 91 |
| 1.31 | 1.16 | 88 | 0.88 | 0.80 | 91 |
| Average | | 90 | | | 91 |

#### Table III. Recovery of Lactonitrile Added to Plasma in Presence of Added Cyanide and Thiocyanate

| Cyanide | | | Thiocyanate | | | Lactonitrile | | |
|---|---|---|---|---|---|---|---|---|
| Added, γ | Found, γ | Recovery, % | Added, γ | Found, γ | Recovery, % | Added, γ | Found, γ | Recovery, % |
| 0.04 | 0.05 | 125 | 0.13 | 0.14 | 108 | 0.43 | 0.38 | 88 |
| 0.62 | 0.62 | 100 | 0.25 | 0.26 | 104 | 2.12 | 1.86 | 88 |
| 1.24 | 1.28 | 103 | 0.51 | 0.56 | 110 | 4.26 | 3.78 | 89 |
| 0.04 | 0.05 | 125 | 0.51 | 0.51 | 100 | 4.26 | 3.81 | 87 |
| 0.62 | 0.62 | 100 | 0.25 | 0.25 | 100 | 2.12 | 1.91 | 90 |
| 1.24 | 1.24 | 100 | 0.13 | 0.12 | 92 | 0.43 | 0.39 | 91 |
| 0.04 | 0.05 | 125 | 0.13 | 0.13 | 100 | 0.43 | 0.40 | 93 |
| 0.62 | 0.62 | 100 | 0.51 | 0.54 | 106 | 4.26 | 3.80 | 89 |
| 1.24 | 1.24 | 100 | 0.25 | 0.26 | 104 | 2.12 | 1.90 | 90 |
| Average | | 109 | | | 103 | | | 89 |

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# Spectrophotometric Determination of Glyoxal Bis(2,4-dinitrophenylhydrazone), a Derivative of Glycolaldehyde

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Interest in the possibility of the occurrence of glycolaldehyde as an intermediate in the enzymatic oxidation of carbohydrates in certain animal tissues stimulated a spectrophotometric study of glyoxal bis(2,4-dinitrophenylhydrazone), a derivative of glycolaldehyde. The solvent was alkaline acetone. The procedure designed was applicable for the determination of glycolaldehyde in microgram quantities. The measurement obeyed Beer's law, the absorption at 600 m$\mu$ was stable, and the standard error for the estimate was 0.002.

ALTHOUGH the spectral absorption of glyoxal bis(2,4-dinitrophenylhydrazine) in sodium ethylate had been described (4), there was no mention of the absorbance coefficient or the nature of the conformance of the measurement to Beer's law. While comparing the quality of a synthetic product of glycolaldehyde with that of a commercial sample, it was observed that the absorbance of the derivative with 2,4-dinitrophenylhydrazine in freshly prepared alkaline acetone not only differed from that of other carbonyl compounds tested but was maximal at a wave length (600 m$\mu$) longer than that previously observed (575 m$\mu$) in sodium ethylate. This latter difference in absorption thereby reduced the possibilities for interference from other carbonyl compounds, the phenylhydrazones of which absorb near 440 m$\mu$. The present report describes the absorption in alkaline acetone and the linear relationship between concentration of the derivative and absorbance when the amounts of the derivative measured were between 5 and 45 $\gamma$. As previously observed by others (5) the reaction product with 2,4-dinitrophenylhydrazine appeared to be the osazone. Thus, applications of the measurement were limited to the derivative of either glycolaldehyde or glyoxal alone, but not selective for one in the presence of the other.

## EXPERIMENTAL

The glycolaldehyde used had two sources; one was a commercial product (Metro Industries) and the other synthetic.
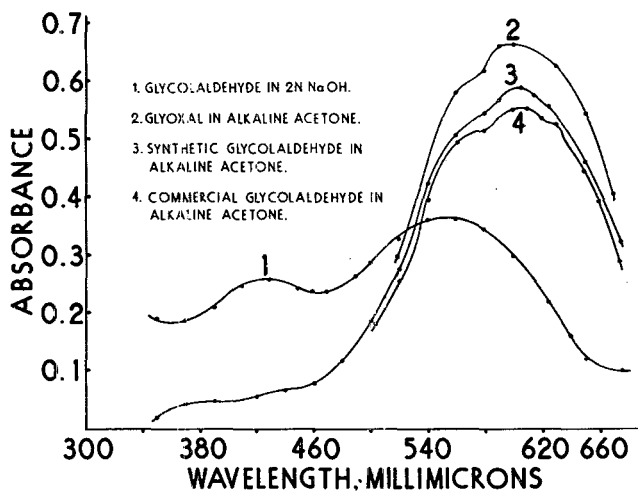


Figure 1. Absorption spectra of 2,4-dinitrophenylhydrazine derivatives of glycolaldehyde and glyoxal

Table I. Observed and Calculated Values of Absorbance at 600 m$\mu$ for Varying Concentrations of Osazone of Glycolaldehyde

| Concentration, $X$ | Absorbance, $Y$ | $Y_m$[a] | $Y_c$[b] |
|---|---|---|---|
| 5 | 0.174 | 0.176 | 0.179 |
| | 0.170 | | |
| | 0.184 | | |
| | 0.177 | | |
| 10 | 0.362 | 0.359 | 0.358 |
| | 0.372 | | |
| | 0.356 | | |
| | 0.346 | | |
| 15 | 0.528 | 0.519 | 0.538 |
| | 0.502 | | |
| | 0.522 | | |
| | 0.522 | | |
| 30 | 1.11 | 1.11 | 1.07 |
| | 1.09 | | |
| | 1.09 | | |
| | 1.10 | | |
| 45 | 1.57 | 1.57 | 1.61 |
| | 1.58 | | |
| | 1.55 | | |

[a] Mean absorbance at indicated concentration.
[b] Calculated (3) values of $Y$, when slope, $m$, and intercept, $b$, are calculated as

$$m = \frac{\Sigma XY - \Sigma X \frac{\Sigma Y}{N}}{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}$$

and

$$b = \frac{\Sigma Y - (m)(\Sigma X)}{N}.$$

In order to prepare the derivative of the commercial product, the glycolaldehyde was dissolved in water so that the concentration was approximately 0.2% and an excess of 2,4-dinitrophenylhydrazine in 1N hydrochloric acid was added. A typical preparation after four crystallizations melted with decomposition between 280° and 295° C. (authentic 290° to 300° C.). When this product was analyzed for total nitrogen (2), two determinations showed 25.76 and 25.69% of nitrogen. The percentages of nitrogen obtained were lower than that of the osazone (theory, 26.79%) but higher than that of the hydrazone (theory, 23.30%). (Presumably the deviation of the observed value for per cent nitrogen from that of the theoretical for the osazone was due to incomplete reduction or loss during manipulation.) However, the spectral absorption of several preparations of the derivative of reagent glyoxal with 2,4-dinitrophenylhydrazine were identical to those of glycolaldehyde. Typical spectra for these (Figure 1) indicate that the absorbance in the alkaline acetone was indistinguishable for the derivative from the two sources. The Beckman spectrophotometer, Model DU, was used throughout these studies.

The synthetic glycolaldehyde was prepared for analysis by treating 100 mg. of serine in a 20-ml. beaker with 2.5 ml. of Clorox (sodium hypochlorite) for about 2 minutes. More than 2.5 ml. of Clorox lowered the yield of glycolaldehyde and reagent sodium hypochlorite of similar concentration did not improve the yield. The mixture in the beaker was neutralized by the dropwise addition of concentrated hydrochloric acid and all solvent was evaporated in a desiccator over sodium hydroxide pellets and phosphorus pentoxide. The dry residue was dissolved in water and treated with 2,4-dinitrophenylhydrazine as described above. The yield, determined spectrophotometrically, was approximately 10% of the theoretical.

That the aldehyde originally synthesized was glycolaldehyde and not glyoxal (not distinguishable as osazones) was evident from the reaction with $\alpha$-naphthoresorcinol. The spectral

characteristics of the dissolved product in a mixture of 5 ml. of 1N sodium hydroxide and 95 ml. of acetone appear in Figure 1. Here also are shown the data for the derivative of the synthetic product dissolved in 2N (aqueous) sodium hydroxide. Although not shown, the data for the osazone of the commercial glycolaldehyde were identical in 2N (aqueous) alkali. In alkaline acetone, spectral curves of the derivative of the synthetic product and those of the product with reagent glyoxal, like those of the commercial product, were superposable.

As the extinction coefficient for the solute at maximum absorption was greater in the solution of acetone containing 1N sodium hydroxide, the degree to which the absorption at 600 m$\mu$ obeyed Beer's law was examined. The data in Table I indicate the applicability of the measurement for quantities of the derivative between 5 and 45 $\gamma$. For all concentrations shown, the absorption remains unchanged within 25 minutes. In Table I, the standard error of the estimate is 0.002—i.e., standard error of the estimate, $s = \sqrt{\dfrac{\Sigma d^2}{n-2}}$, where $d$ is the difference between value, $Y_c$ obtained for a given $x$ and the mean value, $Y_m$, for three or more experimental values corresponding to the given $X$ value (3).

Several derivatives of some carbonyl compounds known to occur in animal tissues were examined for absorbance in order to ascertain, to some degree, the extent to which the absorbance for the glycolaldehyde derivative was characteristic. Glucose, pyruvic, oxalacetic, and $\alpha$-ketoglutaric acids showed maximal absorbance in the alkaline acetone solution at 540, 420, 440, and 400 m$\mu$, respectively. The derivatives of acetone and acetoacetic acid both absorbed at 440 m$\mu$. In addition, when glycolaldehyde was added to chick embryo liver, the osazone chromatographed (1) on paper showed a chromatographic mobility like that of the derivative of the reagent material. When the spot was leached from the paper, the spectral characteristics in acid acetone were like those of the pure derivative.

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# Separation of Alcohols as Their Alkyl Hydrogen Phthalates by Partition Chromatography

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In an investigation of the alcohols in apple storage volatiles, it was found necessary to separate mixtures of alkyl hydrogen phthalates extracted from non-aqueous mixtures of other components. As a result a direct visual method of partition chromatography on silica gel was developed for separating derivatives of the normal aliphatic alcohols ($C_1$ to $C_8$) from a multicomponent mixture. The method should be especially useful for mixtures in which compounds other than alcohols interfere with the preparation or isolation of the more usual alcohol derivatives.

THE chromatographic separation of alcohols as their colorless derivatives, the 3,5-dinitrobenzoates, has been made practical by the application of various techniques for locating the relative positions of these compounds on a column. White and Dryden (11) separated the 3,5-dinitrobenzoates of various aliphatic alcohols on a mixture of silicic acid and diatomaceous earth made fluorescent to ultraviolet light with rhodamine 6 G. The individual bands on the column were evidenced by a diminution of fluorescence. Holley and Holley (4) have used a modification of this method for the identification of alcohols in dilute aqueous solutions. The 3,5-dinitrobenzoates were separated on a 2 to 1 silicic acid and Celite column with petroleum ether as the developer, and eluate fractions were examined spectrophotometrically at 240 m$\mu$. Rice, Keller, and Kirchner (9) reported the successful separation of microgram quantities of 3,5-dinitrobenzoates by filter paper strip chromatography. The strips were reacted with 1-naphthylamine to produce orange or reddish colored spots. Kariyone and Hashimoto (5) have separated lower alcohols by the paper partition chromatography of their potassium xanthogenates with the spots exhibiting a brown luminescence when viewed under ultraviolet light. Momose and Yamada (6) have reported the separation of 3,6-dinitrophthalates of alcohols by paper partition chromatography.

Attempts in this laboratory to prepare the 3,5-dinitrobenzoates of small amounts of alcohols present in mixtures with relatively large amounts of water-insoluble esters and carbonyl compounds resulted in derivatives which were completely soluble in the nonaqueous phase. However, the free alcohols present in the mixture were readily converted to their corresponding half esters with phthalic acid as described by Elving and Warshowsky (1) in a procedure for the determination of alcoholic hydroxyl groups. The resulting hydrogen phthalates could then be extracted from the nonaqueous phase with dilute sodium bicarbonate solution and subsequently reconverted to the free hydrogen phthalates of primary and secondary alcohols. In the procedure tertiary alcohols are dehydrated and do not give the desired half esters (1).

The present paper reports the separation of the alkyl hydrogen phthalates of the normal aliphatic alcohols from ethyl through octyl by partition chromatography on silicic acid. Bromocresol green was used as an indicator to follow band positions.

## EXPERIMENTAL

**Preparation of Alkyl Hydrogen Phthalates.** Four grams of finely ground phthalic anhydride was mixed with 1 gram of each of the normal alcohols from methyl through octyl, 5 ml. of toluene was added to all alcohols above butyl, and the mixtures were refluxed for 1 hour. After refluxing, the reaction mixture was shaken with 25 ml. of benzene, filtered to remove excess anhydride, and neutralized with a 5% solution of sodium bicarbonate. The aqueous layer was then washed with two 25-ml. portions of benzene to remove unreacted alcohols and diesters, acidified with dilute hydrochloric acid, and extracted with three 25-ml. portions of chloroform to remove the half esters. The solvent was removed from the combined chloroform extracts by

gentle warming under reduced pressure. Each of the products was initially an oil, although some of the lower half esters became crystalline on standing for several weeks. Those which crystallized were identified by melting point, the others by direct conversion of their alcohol component to the solid 3,5-dinitrobenzoate by the method suggested by Shriner and Fuson (10) for higher esters. The melting points of both the crystalline half esters and the 3,5-dinitrobenzoates derived from them, or from the oils, agreed with those reported in the literature (2, 10). Alkyl hydrogen phthalates prepared from commercial alcohols should be purified by chromatography before use as reference standards. Solution of the half esters in chloroform followed by filtration and evaporation of the solvent effectively removed traces of phthalic acid.

**Preparation of Chromatographic Columns.** The two types of silicic acid slurries used in the chromatographic separations were similar to those developed for the separation of $C_1$ to $C_{10}$ fatty acids by Ramsey and Patterson (7, 8).

A suitable slurry for the $C_5$ to $C_{10}$ column was prepared by mixing 80 grams of silicic acid (Mallinckrodt analytical reagent grade, specially prepared for chromatographic analysis), 48 ml. of methanol, 4 ml. of bromocresol green indicator solution (200 mg. of dye dissolved in 25 ml. of methanol), 40 drops of $0.2N$ ammonium hydroxide, and 200 ml. of iso-octane (Phillips Petroleum Co., commercial grade) saturated with 90% methanol.

A suitable slurry for the $C_1$ to $C_4$ column was prepared by mixing 80 grams of silicic acid, 8 ml. of water, 8 ml. of bromocresol green indicator solution (100 mg. of dye dissolved in 25 ml. of water with 1.5 ml. of $0.10N$ ammonium hydroxide added), 8 ml. of $0.020N$ ammonium hydroxide, and 300 ml. of a 1% butanol solution in chloroform (washed with several equal volumes of water).

Both slurries were prepared by adding a solution of the indicator, ammonium hydroxide, and immobile phase (water or methanol) to the silicic acid in a mortar and grinding to assure uniform distribution of the immobile phase and the indicator. The addition of chloroform or iso-octane with stirring then produced a thin, blue-green slurry which could readily be poured into chromatographic columns. It was found convenient to prepare several batches of this material at one time, and to store them in tightly stoppered bottles for future use.

**Chromatographic Separation of Half Esters.** Two sizes of columns were used. The first (19 mm. in inside diameter) was fitted with the silicic acid-iso-octane slurry and packed with 10-pound pressure supplied from a nitrogen tank to a depth of 30 cm. Approximately 20 mg. of each of the eight half esters were mixed and dissolved in 7 ml. of iso-octane (saturated with 90% methanol), the solution was forced into the column under 10-pound nitrogen pressure, and elution was effected with the same solvent. The positions of the yellow bands were measured in millimeters from the top of the packing at effluent volumes of 150 and 400 ml., including the 7 ml. of solvent used in putting the half esters on the column.

A column of identical size was prepared with the silicic acid-chloroform slurry. Twenty milligram of each half ester was put on this column in 7 ml. of a 1% butanol in chloroform solution and developed with the same solvent. The positions of the bands were measured in millimeters from the top at 150-ml. effluent volume.

A smaller chromatographic column (10.4 mm. in inside diameter) was packed with a silicic acid-iso-octane slurry to a depth of 29 cm. as described above for the larger columns. In this case approximately 5 mg. of each half ester was added to the column in 4 ml. of solvent. With this size column, only 35 ml. of effluent volume was required to obtain complete separation into eight clearly distinguishable bands.

The identity of each band was determined by mixed chromatograms with known half esters and by position of the bands as compared with the position of known half esters run alone on an identical column and developed to the same degree—i.e., the same effluent volume. Mixed chromatograms were prepared in the following manner. After the columns were completely developed they were extruded, the bands cut from the column, and the half esters extracted from the bands with several 10-ml. portions of ethyl ether. The ether was removed from the extract under reduced pressure, an approximately equal amount of a known half ester added, and the mixture then dissolved in a few milliliters of the proper solvent and rechromatographed on a new column.

### RESULTS AND DISCUSSION

The alkyl hydrogen phthalates used were not recrystallized because of the difficulty in obtaining crystalline products initially.

Formation of the 3,5-dinitrobenzoate of the corresponding alcohol by direct action with the alkyl phthalate, either crystalline or oil, is taken as proof of identity.

Table I exemplifies the separation obtained with approximately 20 mg. of each of the eight half esters studied on the large column with the two different types of slurries. With water as the immobile phase (silicic acid-chloroform) only methyl, ethyl, and propyl were completely separable; the butyl band was faintly differentiated and all higher half esters were eluted without separation. With methanol as the immobile phase (silicic acid-iso-octane slurry) the half esters of propyl through octyl were easily differentiated, but a clear separation of methyl and ethyl was obtainable only with extensive development.

**Table I. Definition of Bands Obtained with Standard Eluate Volumes in Separation of N-Alkyl Hydrogen Phthalates by Partition Chromatography on Silicic Acid**

| N-Alcohol as Hydrogen Phthalate | Methanol–Iso-octane, effluent volume | | Water-Chloroform[a], effluent volume |
|---|---|---|---|
| | 150 ml. | 400 ml. | 150 ml. |
| Methyl | 10–18 | 32–50 | 42–107 |
| Ethyl | 18–27 | 60–74 | 120–165 |
| Propyl | 30–45 | 88–114 | 175–210 |
| Butyl | 50–71 | 145–175 | 225–285 |
| Amyl | 79–102 | 210–250 | 285– |
| Hexyl | 115–147 | Off column | Off column |
| Heptyl | 170–200 | Off column | Off column |
| Octyl | 226–263 | Off column | Off column |

[a] 19-mm. I.D., depth 30 cm., charged with approximately 20 mg. of each half ester.

To establish whether the individual half esters in a mixture would affect the position of each other on a column, a chromatogram with an eight component mixture was prepared and eight separate chromatograms were prepared, one for each of the single components. For these experiments the small column (10.4 mm. in inside diameter) was used. The multicomponent charge was approximately 40 mg. of half esters, the single charge was approximately 5 mg., and 35-ml. effluent volume was collected in each case. The results (Table II) show that the bands separating from a multicomponent mixture were narrower than those produced by the single half esters. The distance of movement of the center of each band from the top of the column was essentially constant.

**Table II. Comparison of Band Positions of N-Alkyl Hydrogen Phthalates Chromatographed Alone or as Mixtures**

| N-Alcohol as Hydrogen Phthalate | Position of Band[a] in Mixture, Mm. | Position of Band[a] Alone, Mm. |
|---|---|---|
| Methyl | 10–12 | 7–13 |
| Ethyl | 16–19 | 14–21 |
| Propyl | 25–30 | 19–34 |
| Butyl | 35–45 | 28–40 |
| Amyl | 54–64 | 52–76 |
| Hexyl | 81–94 | 77–109 |
| Heptyl | 108–127 | 100–131 |
| Octyl | 137–168 | 130–160 |

[a] Silicic acid with methanol–iso-octane, 10.4-mm. I.D., depth 29 cm., band positions measured at 35-ml. effluent volume.

The method has been in use in the biochemistry laboratory of Purdue University in the separation and identification of components of the volatile substances produced by apples in the storage warehouse (3). As with the aliphatic fatty acids (7, 8) the method is probably limited to the separation of alcohols by

numbers of carbon atoms rather than chain length. The behavior of branched homologs has not been studied.

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# Turbidimetric Determination of Nicotine
## An Analytical Application of Valser's Reagent

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To determine nicotine in the presence of nornicotine, the nicotine is selectively and colloidally precipitated as nicotine iodomercurate in 0.2N sulfuric acid, with to 37 times more nornicotine iodomercurate in solution. A turbidimetric comparison with known amounts of nicotine iodomercurate in suspension allows its estimation. The method is more specific for nicotine than existing total alkaloid methods and is faster than methods that differentiate between nicotine and nornicotine. It is more specific and less time-consuming than the official method.

METHODS for the determination of nicotine have a long history, starting in 1846 with Schloesing (21), who advanced a remarkably good analytical method for nicotine in tobacco that depended on titrating the free bases which were extracted from an ammoniacal aqueous tobacco extract with ether.

The official method of the Association of Official Agricultural Chemists (AOAC) (1) is based essentially on the original work of Bertrand and Javillier (2), as modified by Chapin (4).

Many other methods for nicotine have been published. Kissling (10) suggested a colorimetric procedure based on an iodomercurate precipitate. Subsequently, polarographic (22), polarimetric (6), titrimetric (11), turbidimetric silicotungstate (24), spectrophotometric (25), amperometric (5), and turbidimetric phosphotungstate (16) methods appeared, all of which measured total alkaloids or various mixtures of alkaloids.

A method for determining nicotine and nornicotine (3) by making two steam distillations, one for total alkaloids via the gravimetric silicotungstate method (1) and the other for nicotine only, after nornicotine has been made nonvolatile with nitrous acid, has been suggested. Nornicotine is assumed to be represented by the difference.

A partition-chromatographic method (8) isolates the two main alkaloids, nicotine and nornicotine, in different portions of a column of starch. Each is then eluted separately and titrated. Ammonia interferes with the determination of the nornicotine fraction but can be removed with a long pretreatment over sulfuric acid.

A paper chromatographic method (23) separates and isolates

the alkaloids of tobacco but, in common with most paper chromatographic methods, takes time and lacks precision. A colorimetric method (13) depends upon the two different colors produced by the main alkaloids when treated with a cyanogen bromide reagent. The degree to which each compound interferes with the analysis of the other can be predicted from calibration curves and used to calculate a more correct value.

Nornicotine differs from nicotine only by having an NH group instead of an N.CH3 group in its pyrrolidine ring. Its toxicity has been found to vary greatly from that of nicotine, being both more and less toxic, depending upon the species of test animal (14-16, 18). It occurs in tobacco and tobacco products in fractions of the total alkaloid content that vary from a few per cent in most grades to almost the total amount in tobaccos especially bred for low nicotine content (9).

## EXPERIMENTAL

In a previous paper (20) it was reported that many pure alkaloids and organic bases can be determined quantitatively by measuring the turbidity produced by adding to their solutions a solution of potassium iodomercurate (Valser's reagent). For the routine control of certain pharmaceutical products, this method allows the determination of certain alkaloids and organic bases in the presence of others. For example, dihydrocodeinone and thenylpyramine can be determined in the presence of ephedrine, which forms a soluble complex with the reagent, at the concentrations employed in the assay.

In order to see whether a similar difference in solubilities might allow the preparation of a colloidal nicotine suspension while keeping nornicotine iodomercurate in solution, the following experiments were conducted:

Increasing amounts of pure nicotine were made to react with Valser's reagent in the range of concentration 1 to 10 $\gamma$ of nicotine per milliliter. The absorbances of the resulting suspensions as measured on the Fisher Electrophotometer, filter 525, light intensity B, are plotted against the amount of nicotine in Figure 1.

By treating nornicotine solutions containing amounts increasing from 10 to 500 $\gamma$ per ml., it was found that a concentration of 150 $\gamma$ per ml. must be reached before a measurable precipitate appears.

Repeating the series of nornicotine concentrations but with 8 $\gamma$ per ml. of nicotine added to each, it was found that no copre-

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cipitation takes place below 150 γ per ml. of nornicotine. At the 150 γ per ml. concentration of nornicotine, the increment of absorbance added to that of the 8 γ per ml. concentration of nicotine as iodomercurate amounts to about 25%. Thus, 8 γ per ml. of nicotine, near the top of the straight-line portion of the curve in Figure 1, can be correctly estimated without interference from nornicotine, when the concentration of nornicotine is anywhere from none to 15 times more than that of the nicotine. By rerunning the nicotine solution at a diluted concentration of 4 γ per ml., the bottom of the usable portion of the curve, up to 37 times more nornicotine can be tolerated without interference.
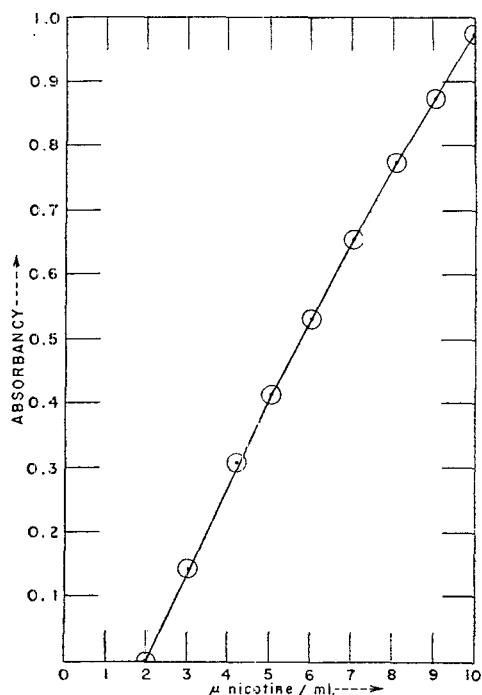


**Figure 1. Relation of amount of nicotine (as iodomercurate) to absorbance**

Table I shows how model mixtures of nicotine and nornicotine are separated into component fractions by distilling an aliquot of a solution containing both alkaloids as in the AOAC method, then assaying the distillate by turbidimetric iodomercurate precipitation and gravimetric silicotungstate precipitation.

**Table I. Nicotine in Model Mixtures with Nornicotine**

| Composition of Mixture, Mg. | | Alkaloid, Mg. | |
| --- | --- | --- | --- |
| Nicotine | Nornicotine | As nicotine (turbidimetric iodomercurate) | AOAC method |
| 100 | 10 | 99.2 | 101.0 |
| 100 | 30 | 99.5 | 107.7 |
| 100 | 70 | 98.5 | 148.3 |
| 100 | 100 | 100.5 | 182.1 |
| 100 | 200 | 99.6 | 290.4 |
| 100 | 1000 | 99.7 (25/250 aliq.) | 1085.6 |
| 100 | 2000 | 98.4 (25/250 aliq.) | 2089.9 |

Additional experiments were made with pyridine and also with ammonia in place of the nornicotine. An amount of pyridine 30 times the amount of nicotine could be tolerated without interference. Ammonium sulfate of a concentration exceeding that of nicotine by a factor of 10,000 can be added without causing interference. Above this concentration ammonium sulfate exerts a slight solubilizing effect as evidenced by decreasing absorbance of the nicotine iodomercurate suspension.

In the course of this work, it was observed that nicotine iodomercurate suspensions are not as stable as the more insoluble iodomercurates of methylhomatropine and thenylpyramine. Glucose, sucrose, and starch were tried as stabilizing agents with the results shown in Figure 2. Since starch yielded suspensions equally stable when compared to the other agents, and, in addition, yielded absorbance values closely approximating the values obtained without a stabilizing agent, it was selected as the most suitable stabilizer, in accord with the findings of Kozu (12) for the turbidimetric silicotungstate method.

At this point the desirability of checking methods of separating the alkaloids from tobacco by distillation became apparent. The prime prerequisite for a turbidimetric determination is, of course, a clear, preferably colorless solution. The distillation procedure used in the official method yields this kind of a nicotine solution but takes several hours to prepare. It was therefore decided to attempt the distillation on a semimicro scale using the Clough apparatus (7) designed for the distillation of ammonia from micro-Kjeldahl digestion flasks. The ground-glass, standard-taper joints of this apparatus made it particularly useful from the standpoints of ease of cleaning, operation, and changing samples.

By distilling identically sized samples from the same batch of tobacco for increasing lengths of time, then assaying the distillate by the gravimetric silicotungstate method, it was established that 98% of the total alkaloids were distilled over in the first 10 minutes. However, to provide a sufficient safety factor for refractory samples, 30 minutes was chosen for subsequent distillations.

**Preparation of Sample.** Tobacco samples are passed through a small Wiley mill. Moisture values (100° C., 3 hours in a forced draft oven) are determined both before and after grinding if it is desired to report the nicotine values on the "as received" basis. Liquid insecticides are weighed in a small weighing bottle, then transferred with the aid of a stream of water into volumetric flasks, diluted to a known volume, mixed, and filtered. Powdered insecticides can be treated like tobacco powders.

**Reagents.** VALSER'S REAGENT. Dissolve 70.0 grams of mercuric iodide ($HgI_2$) in a solution of 50.0 grams of potassium
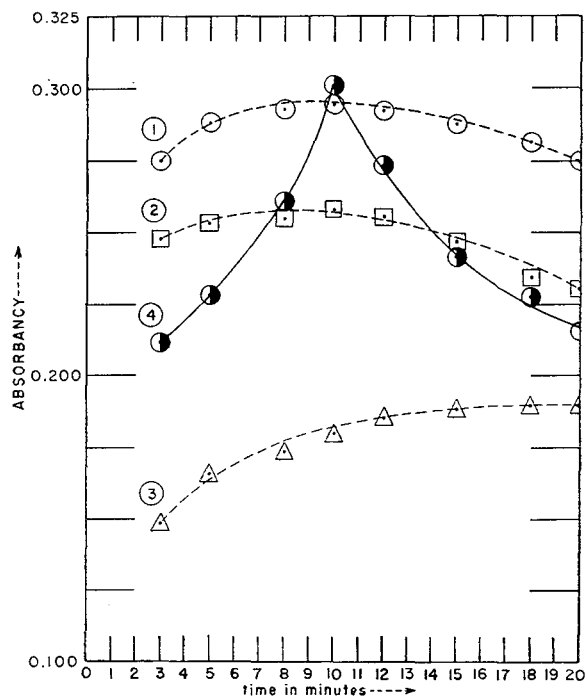


**Figure 2. Stability of nicotine iodomercurate suspensions**

1. Starch as stabilizing agent
2. Sucrose as stabilizing agent
3. Glucose as stabilizing agent
4. No stabilizing agent

iodide (KI) in distilled water. Add 5 ml. of 10$N$ sulfuric acid and dilute to 500 ml. One milliliter of freshly prepared 1% starch solution added to this reagent will indicate the appearance of free iodine, which can be removed by the addition of a few drops of 0.1$N$ sodium thiosulfate.

SULFURIC ACID, 0.2$N$.
SULFURIC ACID, 10$N$.
STARCH SOLUTION, 1%.
NICOTINE SALICYLATE (eudermol).

STANDARD NICOTINE SOLUTION. Distill an amount of nicotine salicylate from a 30% sodium hydroxide solution in the Clough apparatus of a concentration sufficient to yield a distillate which, when diluted to a known volume, will have a concentration of exactly 50.0 γ of nicotine alkaloid per milliliter in 0.2$N$ sulfuric acid.

Procedure. An amount of powdered tobacco or insecticide expected to contain between 7.5 and 14.5 mg. of nicotine is placed in a 100-ml. ℱ Kjeldahl flask with 30 ml. of 30% sodium hydroxide. It is steam-distilled as fast as possible from the micro-Kjeldahl distilling apparatus into a 250-ml. volumetric flask containing 5 ml. of 10$N$ sulfuric acid. The volume of the tobacco suspension in the distilling chamber should gradually decrease to about 10 ml. during the distillation. If it does not, a micro-Bunsen burner can be used to bring this condition about with controlled heat directed at the bottom of the sample-containing flask. The tip of the condenser should be immersed in the acid. The distillation is stopped at 30 minutes or just before the receiver's calibration mark is reached, whichever comes first. The distillate is diluted to volume with distilled water and mixed.

#### Table II. Determination of Nicotine in Tobacco and Insecticides

| Sample | Nicotine (Turbidimetric Iodomercurate), % | Total Alkaloids (AOAC Method), % | Difference % |
|---|---|---|---|
| TRP-1 | 2.10 | 2.28 | 0.18 |
| TRP-2 | 1.75 | 1.94 | 0.19 |
| TRP-4 | 3.30 | 3.62 | 0.32 |
| TRP-5 | 1.08 | 1.19 | 0.11 |
| TRP-10 | 4.00 | 4.34 | 0.34 |
| TRP-11b | 5.15 | 5.50 | 0.35 |
| TRP-5s | 0.26 | 0.31 | 0.05 |
| TRP-7s | 0.56 | 0.63 | 0.07 |
| TRP-14s | 1.05 | 1.27 | 0.22 |
| BTA-1 | 5.19 | 5.65 | 0.51 |
| | 5.11 | 5.64 | |
| | 5.15 | 5.65 | |
| | 5.16 | | |
| | 5.15 | | |
| | 5.12 | | |
| Insecticide 1[a] | 33.25 | 39.85 | 6.38 |
| Insecticide 2[b] | 1.40 | 1.91 | 0.51 |
| Nicotine alkaloid[c] | 95.13 | 95.17 | 0.04 |

[a] Labeled 40% nicotine.
[b] Labeled 1.60% nicotine.
[c] c.p. labeled 95% nicotine.

Aliquots of 3.00 ml. in triplicate of distillate are placed in cylindrical, 25-ml. absorption cells 23 mm. in diameter. Aliquots of 3.00 ml. of standard nicotine solution, also in triplicate, are placed in other cells. A blank cell containing 3.00 ml. of 0.2$N$ sulfuric acid is prepared.

Two milliliters of 1% starch solution and 17.00 ml. of water are added to each tube. The tubes are rubber-stoppered and mixed by inversion.

At 30-second intervals 1.00 ml. of Valser's reagent is added to each cell in succession and mixed with the stopper-inversion technique. Exactly 10 minutes later (timed from the first addition of precipitant) the absorbance of each cell is read at 30-second intervals, in the same order as precipitated, with the blank tube containing no nicotine set at zero absorbance. Filter 525, light intensity B, on the Fisher Electrophotometer was found satisfactory.

When the absorbance values found fall on the straight-line portion of the calibration curve (4 to 8 γ of nicotine per milliliter) the arithmetical mean of the triplicate determinations is used to calculate per cent nicotine according to the following formula:

$$\% \text{ nicotine} = \frac{0.00015 \times A_x \times V \times 100}{\text{sample weight} \times [0.529(A_k - A_x) + A_k] \times 3}$$

where
0.529 $(A_k - A_x)$ is an empirically derived factor to correct for the solubility of nicotine iodomercurate at 26° C.
$A_x$ = absorbance of the sample suspension

$A_k$ = absorbance of the standard nicotine solution (equivalent to 0.000150 gram of nicotine)
$V$ = final volume of distillate (250 ml.).

### RESULTS

Using this procedure a series of tobacco samples from various sources and commercial nicotine-containing insecticides was analyzed. In Table II the results are compared with the percentage of total alkaloids according to the official AOAC method, gathered at the same time. The difference between the data collected by the two methods, presumably nornicotine, is tabulated in the third column. The difference may also include anabasine and other nicotine transition compounds.

### DISCUSSION

This method should be useful in determinations of nicotine in tobacco where time is a factor, such as the determination of nicotine in tobaccos especially bred for low nicotine content, where the high content of nornicotine must be eliminated as an interfering compound, and in the quick estimation of possible toxicity in insecticides, providing that purity of its nicotine alkaloid content is the deciding factor.

### ACKNOWLEDGMENT

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# Stability of Colorimetric Reagent for Chromium, s-Diphenylcarbazide, in Various Solvents

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The deterioration and sensitivity of s-diphenylcarbazide were studied in a variety of solutions involving seven organic solvents. Nonaqueous ethyl acetate and acetone solutions were stable for months; methyl ethyl ketone, methyl Cellosolve, and 2-propanol solutions were usable for 1 to 2 weeks. Aqueous solutions and solvents tending to be basic, such as methanol, ethyl alcohol, and those containing traces of water or basic impurities, do not make good solvents for stock solutions of the colorimetric reagent.

A WIDELY used colorimetric reagent for chromium is s-diphenylcarbazide (1,5-diphenylacarbohydrazide). However, there has been no standard method of preparing a solution of this reagent. Cazeneuve (3) used the powdered compound or an acetic acid solution; Moulin (8) used an acetic acid–ethyl alcohol solution; Rowland (9) and Feigl (6) used ethyl alcohol solutions; Graham (7) used a sulfuric acid–ethyl alcohol solution; and Sandell (11) used a 1 to 1 acetone-water solution. Davis and Bacon (4) briefly discussed the sensitivity and life of the reagent stating that alcohol solutions lose sensitivity within 2 days, acetic acid solutions deteriorate rapidly, and acetone solutions seemed to hold up for longer periods of time. As a rule, fresh solutions were required, whenever quantitative measurements were to be taken.

More recently Ege and Silverman (5) proposed a stable solution in which 0.25 gram of s-diphenylcarbazide and 4.0 grams of phthalic anhydride are dissolved in 100 ml. of 95% ethyl alcohol. The reagent was usable for much longer periods of time and was adaptable for filter paper impregnation (12).

During the course of a recent chromium study (13) a 1 to 1 acetone-water solution of the colorimetric reagent was used. It was noticed that analytical quality acetone from different chemical companies gave solutions of varying stability. The least stable were solutions made with acetone having a slightly basic reaction. These would darken within a short time and when reacted with sexivalent chromium would give low color intensities. However, solutions made from acetone having a slightly acidic reaction were usable for longer periods of time. The chemical companies concerned attributed the basicity to traces of ammonia and the acidity to traces of propionic acid, although traces of formic and acetic acids are more probable.

### EXPERIMENTAL

To study the phenomenon further, duplicate solutions of diphenylcarbazide in various types of acetone were made. One set of the solutions was kept in a refrigerator at 4° C. It was exposed to light only for the short time necessary to withdraw 3-ml. portions of each sample. These portions were used for measuring the absorbance of the solution itself, following which 0.5 ml. of each portion was added to 5 γ of sexivalent chromium in 10.0 ml. of 0.2N sulfuric acid, and the resulting color was measured. The unused part of the portions was discarded and not returned to the original containers. The duplicate set was studied similarly but was kept on top of a reagent shelf clearly exposed to room light (fluorescent) and temperatures. Readings were taken at hourly intervals for the first week, daily for the first week, and weekly thereafter. Later, solutions of the colorimetric reagent in six other organic solvents were studied in the same manner. In all, some 30 solutions were studied.

All solutions were 0.25% in s-diphenylcarbazide, and all measurements were made with a Beckman Model DU spectrophotometer at 540 mμ and using 10-mm. cells. Individual determinations could be repeated with a precision of 2%. The "pH" of the acetones were measured with a Beckman pH meter. However, the diphenylcarbazide itself serves as an indicator. In slightly basic solutions it assumes its basic color and turns light red within an hour or two. The acidic solutions remain slightly yellow or colorless until deterioration takes place.

### DISCUSSION

The stability of the solutions of the diphenylcarbazide was measured in terms of the absorbance of the discoloration (or darkening) developed by the solution with time and the absorbance of the chromium diphenylcarbazide color obtained when 0.5 ml. of the same solution was reacted with 5 γ of sexivalent chromium in 10 ml. of 0.2N sulfuric acid. Figures 1 and 2 give curves typical of the results obtained from the unrefrigerated samples. The refrigerated samples gave slightly better results, but they followed the same general patterns as their unrefrigerated duplicates. If drawn, the curves of the refrigerated samples would closely parallel those of their respective unrefrigerated samples for the first few days, being displaced slightly to the right. In 1 to 2 weeks the differences would become small, and the curves would tend to merge. Hence the general patterns of stability are dependent more upon the type of solution than they are on refrigeration.

**Discoloration of Diphenylcarbazide Solutions.** When freshly made, the diphenylcarbazide solutions are water white to slightly yellow in color. As the solutions age, they become yellow-brown to orange-red depending on the type of solvent and the amount of deterioration. Presumably, the diphenylcarbazide is oxidized to the carbazone. However the reaction is not completely understood. Sandell (10) states that sexivalent chromium does not react with pure diphenylcarbazone while Bose (2) states that it
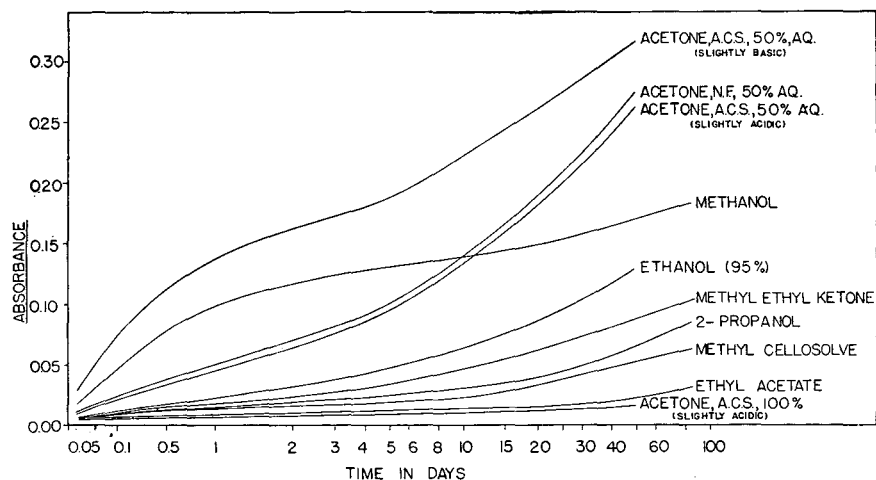


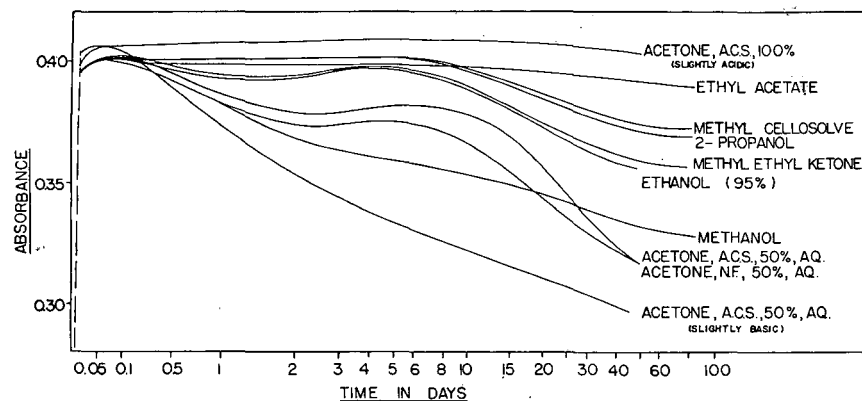**Figure 1. Discoloration of s-diphenylcarbazide solutions with time**

**Figure 2.   Effect of time on sensitivity of s-diphenylcarbazide solutions**

does.  In any event, the reagent does lose sensitivity as it discolors.

The 1 to 1 aqueous solution of the colorimetric reagent made with analytical quality acetone having a slightly basic reaction (top curve, Figure 1) discolored rapidly, due possibly to a basic catalysis of the oxidation of the diphenylcarbazide to the diphenylcarbazone (14).  The same type of solution, using acetones which conformed to National Formulary and AMERICAN CHEMICAL SOCIETY specifications and which had slightly acidic reactions, did not discolor rapidly for the first few days.  However, after this initial period they deteriorated at approximately the same rate.

Nonaqueous solutions proved to have greater stability.  A solution of the colorimetric reagent in acetone conforming to ACS specifications (slightly acidic) was stable for months. A solution (not shown) prepared from acetone, purified by drying over anhydrous calcium sulfate, followed by distillation from phosphorus pentoxide (1), and kept in a refrigerator, discolored only slightly and gave satisfactory results after a 2 year period. Ethyl acetate also made a solution which was stable and usable for as much as 18 months after the period of study shown here. Methanol solutions were unsatisfactory, while ethyl alcohol solutions could be used for approximately a week.  Solutions with methyl ethyl ketone, 2-propanol, and methyl Cellosolve (ethylene glycol monomethyl ether) were usable for approximately 2 weeks.

Except for acetone, no attempt was made to obtain a high purity product.  The acetone having ACS specifications and having a slightly basic reaction was redistilled, but a center fraction gave essentially the same results.  The addition of a drop of 6N sulfuric acid increased the stability somewhat, but the discoloration followed the general pattern of the aqueous acetone solutions.  The methanol, 2-propanol, and ethyl acetate meet ACS specifications for analytical reagents.  The ethyl alcohol was redistilled over potassium hydroxide made according to United States Pharmacopeia, and the methyl Cellosolve was redistilled from technical grade stock.

**Absorbance of Color Developed with Chromium.**  The absorbance of the chromium diphenylcarbazide color varied inversely with the amount of discoloration developed by the solution of the reagent (Figures 1 and 2).  Upon aging, the aqueous acetone solutions reacted with chromium to give colors with low absorbance, while the slightly acidic nonaqueous, acetone solutions gave colors having consistently high absorbances.  The ethyl acetate solutions also gave consistent, although slightly lower, absorbances.

As a rule the freshly made solutions gave the highest absorbance values.  There was a tendency for the aqueous and ethyl alcohol solutions to drop relatively sharply in the first 1 or 2 days, level off for 1 to 2 weeks, and then gradually taper off to lower and lower color intensities.  It is advisable to standardize

all solutions carefully in terms of time, as some lose sensitivity even within the first few hours.  Once sensitivity has been lost it cannot be regained even with excess reagent (4).

### SUMMARY

The stability of solutions of s-diphenylcarbazide, colorimetric reagent for chromium, is greater in nonaqueous organic solvents than it is in the solutions containing water.  As the solutions of the reagent age, they tend to become yellow-brown to orange-red and the sensitivity of the reagent varies inversely with the amount of discoloration developed.  Once sensitivity has been lost it cannot be regained even with excess reagent.  Therefore solutions which become discolored should be discarded or recalibrated.

Ethyl acetate, high purity acetone, or acetone meeting ACS specifications which does not have a tendency to be basic, can be used to make solutions which will be stable for months and give chromium diphenylcarbazide colors of consistently high absorbance.  Methyl ethyl ketone, methyl Cellosolve, 2-propanol, and other similar types of organic solvents make solutions usable for 1 to 2 weeks.  In all probability the elimination of traces of water, basic substances, and oxidizing agents would make these and similar solutions stable for still longer periods of time.  The use of glacial acetic acid–ethyl alcohol solutions and phthalic anhydride–ethyl alcohol solutions is in partial agreement with these principles, since these solutions are acidic and tend to restrict the chemical activity of the water that is present.  Refrigeration of the solutions helps, but the refrigeration effect is minor compared to the effect of the type of solvent used.

Substances tending to be basic, such as methanol, ethyl alcohol, and those containing traces of water or basic impurities, do not make good solvents for stock solutions of the colorimetric reagent.  Aqueous solutions of the reagent should be avoided.

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# 97. Tris(hydroxymethyl)aminomethane (2-Amino-2-hydroxy-methyl-1,3-propanediol)

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HO—CH₂
|
HO—CH₂—C—NH₂
|
HO—CH₂
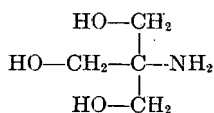
**Structural formula for tris(hydroxymethyl)amino-methane**

TRIS(hydroxymethyl)aminomethane can be crystallized from isopropyl alcohol containing about 20% water. The resulting crystals are needles elongated parallel to the c axis, which may not show complete development of the end faces. Several commercial samples have shown the complete complement of faces mentioned below.
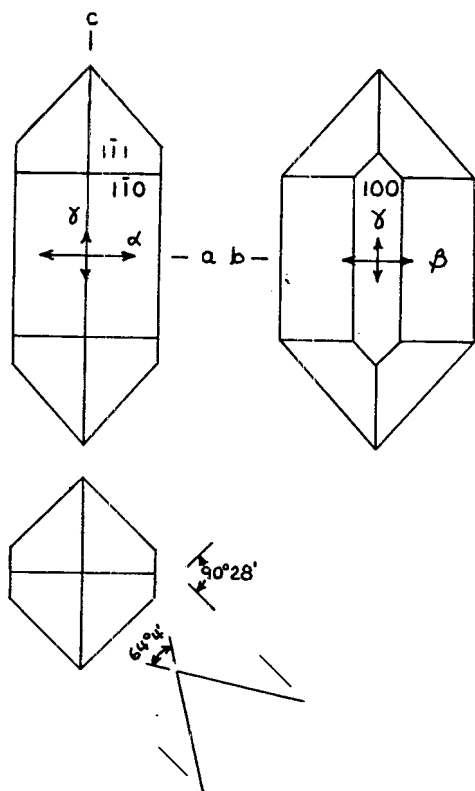


**Figure 1. Orthographic projection of a typical crystal of tris(hydroxymethyl)aminomethane**

The behavior of this material on fusion is unusual. When the crystals are heated on a microscope hot stage as in a melting point determination, there is a sharp loss of birefringence at 135° C. but without loss of external form. If the temperature is lowered while the material is in this state, the orientation of the

resulting crystals bears no relation to the shape of the original crystals. The final melting point or complete reduction to a melt at 172–173° C. is sharp. It is this final figure that is recorded in the literature as the melting point of this compound.

### X-Ray Powder Diffraction Data

| $d$ | $I/I_1$ | $h\,k\,l$ | $d$(Calcd.) |
|---|---|---|---|
| 6.24 | 0.20 | 110 | 6.27 |
| 5.82 | 0.07 | 101 | 5.84 |
| 4.87 | 1.00 | 111 | 4.88 |
| 4.39 | 0.53 | 200 | 4.41 |
| 3.90 | 0.33 | 002, 210, 120 | 3.90, 3.95, 3.97 |
| 3.52 | 0.20 | 211, 121 | 3.52, 3.53 |
| 3.31 | 0.33 | 112 | 3.31 |
| 2.921 | 0.20 | 300 | 2.940 |
| 2.782 | 0.07 | 122 | 2.782 |
| 2.630 | 0.27 | 131 | 2.643 |
| 2.490 | 0.13 | 013, 103 | 2.495, 2.482 |
| 2.436 | 0.07 | 230 | 2.459 |
| 2.397 | 0.13 | 113 | 2.401 |
| 2.298 | 0.13 | 132 | 2.285 |
| 2.266 | 0.07 | 312, 023 | 2.270, 2.211 |
| 2.176 | 0.07 | 123, 140 | 2.145, 2.155 |
| 2.132 | 0.07 | 401 | 2.122 |
| 2.061 | 0.07 | 322, 141 | 2.050, 2.007 |
| 2.008 | 0.07 | 240 | 1.985 |
| 1.946 | 0.07 | 033 | 1.954 |
| 1.900 | 0.07 | 402, 104 | 1.919, 1.904 |
| 1.854 | 0.07 | 332 | 1.840 |

The x-ray powder diffraction data were obtained using a camera 114.6 mm. in diameter and chromium radiation with vanadium filter. A wave-length value of 2.2896 A. was used in the calculations.

CRYSTAL MORPHOLOGY
  Crystal System. Orthorhombic.
  Form and Habit. Needles elongated parallel to c and showing the prism {110}, bipyramid {111}, and usually a small macropinacoid {100}.
  Axial Ratios. $a:b:c = 0.9921:1:0.8774$.
  Interfacial Angles (polar). 110 ∧ 1̄10 = 89° 32′ 111 ∧ 11̄1̄ = 115° 56′·(x-ray).

X-RAY DIFFRACTION DATA
  Cell Dimensions. $a = 8.82$ A., $b = 8.89$ A., $c = 7.80$ A.
  Formula Weights per Cell. 4.
  Formula Weight. 121.14.
  Density. 1.353 grams per cc. 1.318 grams per cc. (calculated)
  Space Group. $P2_12_12_1$.

OPTICAL PROPERTIES
  Refractive Indices (5893 A., 25° C.). $\alpha = 1.514$, $\beta = 1.540$, $\gamma = 1.548$.
  Optic Axial Angle. $2V = (-)\ 57°$ (calculated from $\alpha$, $\beta$, and $\gamma$).
  Optic Axial Plane. 010.
  Acute Bisectrix. $\alpha = a$.
  Molecular Refraction. $\sqrt[3]{\alpha\beta\gamma} = 1.534$; $R = 28.6$ (observed), 28.7 (calculated).

FUSION DATA. Tris(hydroxymethyl)aminomethane loses birefringence sharply at 135° C., but maintains its external form until this also is lost at 172–173° C. On cooling, the melt crystallizes as needles having parallel extinction and .negative elongation. If the solidified melt is reheated, the same sequence of events of loss of birefringence and final melting is followed, although at somewhat lower temperature.

# AIDS FOR THE ANALYST

## Inexpensive Glass Chamber for One- and Two-Dimensional Ascending Paper Chromatography

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INEXPENSIVE plate glass chambers for ascending paper chromatography may be constructed from materials readily available in the laboratory. The total time required for the completion of six chambers was several days; the last chamber was completed in less than an hour. Each chamber cost $2.80, about one tenth the cost of a commercial chamber purchased previously. The homemade chambers, the dimensions of which are 12 × 12³/₈ × 24 inches, have stood up well in a year and a half of use and have several advantages over the relatively expensive commercial cylinders.

### ASSEMBLY OF CHAMBER

The following materials are required for a single chamber. Five single-weight window panes (12 × 24 × ³/₁₆ inch), 144³/₈ inches of 1-inch white adhesive tape, ¹/₂ ounce of Seal All fast-drying plastic cement (Allen Products Corp., Detroit 34, Mich.), and 48¹/₄ inches of All State rubber sponge weather stripping (³/₈ × ¹/₄ inch).
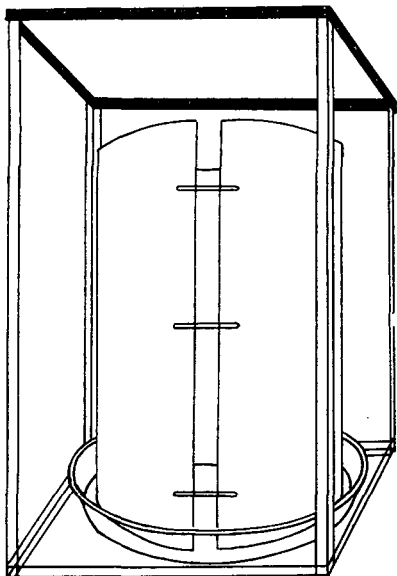


**Figure 1**

**Construction.** All glass surfaces are cleaned with acetone prior to cementing and weather stripping. One pane of window glass is cut into two, using a glass-cutting tool, to make the top and bottom sections of a chamber (Figure 1). The other four window panes are left intact, to form the four sides of the chamber.

One side is attached by cement, followed immediately by adhesive tape, to the 12-inch length of the bottom. The next side pane is attached in similar fashion to the 11⁵/₈-inch base, flush against the previously attached side and at right angles to it. This side pane will protrude ³/₁₆ inch beyond the 11⁵/₈-inch base, the thickness of the glass. The third side pane is attached similarly along the 12-inch base, flush with the ³/₁₆-inch protrusion. The fourth pane is attached similarly along the 11⁵/₈-inch base and flush over the edges of the two sides at right angles to them. If cementing and application of the adhesive are done properly, a square chamber is obtained with over-all dimensions of 12 × 12³/₈ × 24 inches. Weather stripping is applied to the edges of the top pane, which fit flush with the chamber, and is

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permitted to overhang ¹/₈ inch on all sides. Any exposed cement is covered with fast-drying plastic cement to provide tolerance in the fitting.

The solvent mixtures were placed in 10-inch borosilicate glass pie plates which were set on the bottom of the chambers. The size of the pie plate was determined by the 7.5-inch diameter of the filter paper cylinder. The height of this cylinder is 18¹/₄ inches, and the 22¹/₂-inch length of the paper forms the circumference; when the 18¹/₄-inch length forms the circumference, the diameter is 6 inches and the height is 22¹/₂ inches. The edges of the paper cylinders are kept from overlapping by glass hooks. The size of the borosilicate plate made its insertion and removal from the bottom of the commercial circular chamber (12 × 24 inches) very difficult. No such difficulty was experienced with the square chamber.

Although an air-tight chamber is recommended for paper chromatography, it may lead to undesirable results if a constant-temperature room is not available. The time required for the ascent of the solvent mixture is lengthy (24 to 48 hours), and a considerable temperature differential exists between day and night. The night, with its lower temperature, causes an appreciable condensation of the volatile solvent mixture on the sides of the chamber and on the filter paper cylinder. This results in "sweating" and solvent-logging of the filter paper, which causes either the obscuring or the apparent disappearance of the solvent front, with its attendant lower $R_f$ values and poor separation. While the homemade chamber is practically airtight, a certain amount of ventilation through the weather stripping allows the escape of sufficient volatile solvent to prevent undue sweating and condensation without impairing the efficiency of the analysis. The solvent mixture of 1-butanol, acetic acid, and water (4:1:1 by volume) produced sweating and the disappearance of the solvent front in the circular chamber. The solvent mixture of phenol-water (5:1 by volume) caused sweating only when the temperature differential was large. Occasional sweating was encountered when the former solvent mixture was used in the square chamber, but significantly the solvent front was retained up to the top of the 22¹/₂-inch paper cylinder. Neither sweating nor loss of solvent front occurred when the phenol-water solvent was used in the square chamber.

The results obtained by the use of the chambers have been excellent and reproducible. Workers who had access to both the commercial and the homemade types exhibited a decided preference for the latter.

## Desalting Amino Acid Solutions by Ion Exchange

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THE presence of inorganic salts in extracts of biological materials constitutes a serious obstacle in the separation of amino acids by paper chromatography. A number of procedures have been reported for the removal of salts from such solutions (1, 3, 5, 6); however, the need for a simple routine procedure still existed. The following method has been developed in which both amino acids and cations (sodium, potassium, magnesium, and calcium) are first adsorbed on Dowex 50 (hydrogen form) and the majority of the amino acids are subsequently eluted free of the inorganic salts with 0.8N hydrochloric acid in 55% ethyl alcohol. Fifteen amino acids are recovered quantitatively in this eluate; four additional compounds are recovered from the column with 6N hydrochloric acid (aqueous) after the selective elution of sodium chloride with 1N hydrochloric acid (aqueous).

While the procedure has been utilized primarily for the sepa-

ration of small quantities of radioactive amino acids formed by tissue preparations in physiological salt solutions in vitro, it also has been applied successfully to perchloric acid extracts of rat tissues.

## EXPERIMENTAL

Dowex 50 resin (200- to 400-mesh, 12% cross-linked) was prepared in the hydrogen form according to the directions of Hirs, Moore, and Stein (2). Before use batches of this resin were washed with 55% ethyl alcohol until the eluates were no longer turbid. Resin columns, 0.8 cm. in diameter and 5 cm. in height, were prepared from aqueous suspensions of this resin. The appropriate glass tubing sealed onto the bottom of 50- or 100-ml. Erlenmeyer flasks provided convenient columns for this operation.

Samples of amino acids or tissue extracts containing 25 mg. of sodium chloride or potassium chloride, with or without smaller amounts of magnesium or calcium ions, dissolved in 10 to 20 ml. of water or 0.02N perchloric acid were placed on the columns. The columns were successively eluted with 5 ml. of distilled water, 40 ml. of 0.8N hydrochloric acid in 55% ethyl alcohol, 10 ml. of 1N hydrochloric acid in water, and 10 ml. of 6N hydrochloric acid in water. The elutions were conducted at a flow rate of approximately 1 ml. per minute with the aid of slight air pressure. With a manifold as many as 12 columns have been operated simultaneously with ease.

The eluates were customarily evaporated to dryness under a stream of air in a water bath at 55° C. The samples were neutralized to approximately pH 6.3 prior to preparation of the paper chromatograms. The chromatographic systems of McFarren (4) were employed to separate the amino acids in the hydrochloric acid–ethyl alcohol fraction.

## Table I. Recovery of Amino Acids after Desalting with Dowex 50 (Hydrogen Form)

| Amino Acid | % Recovery |
|---|---|
| Aspartic acid | 103 |
| Glutamic acid | 103 |
| Serine | 101 |
| Threonine | 95 |
| Glycine | 100 |
| Alanine | 101 |
| Methionine | 102 |
| Phenylalanine | 98 |
| Leucine | 91 |
| Isoleucine | 95 |
| Valine | 94 |
| Tyrosine | 97 |
| Proline[a] | 106 |
| Hydroxyproline[a] | 112 |
| Tryptophan[a] | 107 |
| Arginine[a,b] | 109 |
| Histidine[a,b] | 106 |
| Lysine[a,b] | 96 |
| Cystine[a,b] | 102 |

[a] Amino acid recoveries determined gravimetrically on 10-mg. samples. All other amino acid recoveries determined colorimetrically on 1-μ mole samples.
[b] Eluted with 10 ml. of 6N hydrochloric acid as in experimental work.

The recovery of known quantities of the individual amino acids was determined either gravimetrically or colorimetrically with ninhydrin on ammonia-free samples according to the procedure of Troll and Cannan (7). One-micromole samples of each amino acid in the presence of salt were used when the colorimetric method was employed. In the gravimetric trials 10 mg. of each amino acid was similarly employed without overloading the column.

## RESULTS

After a 5.0-ml. water wash to remove anions and neutral compounds, the following amino acids were eluted quantitatively with 40 ml. of 0.8N hydrochloric acid in 55% ethyl alcohol: aspartic acid, glutamic acid, serine, threonine, glycine, alanine, methionine, phenylalanine, isoleucine, valine, tyrosine, proline, hydroxyproline, and tryptophan (Table I). A 10-ml. aliquot of 1N hydrochloric acid in water next eluted the sodium completely as the chloride salt. Four additional amino acids (arginine, histidine, lysine, and cystine) were recovered in a final 10 ml. of 6N hydrochloric acid in water. The latter group was contaminated with potassium, magnesium, and calcium ions when present in the

original solution; no special efforts were made to improve this fraction for the present work. The recovery on all amino acids was considered quantitative within the limits of analytical methods employed.

A column of the above dimensions will permit the isolation of amino acids from 3.0 ml. of an isotonic salt solution. If more salt is present in the extract, the volume of the column should be increased proportionately by expanding the diameter of the column; the volume of the eluates should also be increased proportionately.

As the percentage of ethyl alcohol in the eluting hydrochloric acid solution was increased, the affinity of the resin for the majority of amino acids was decreased relative to the affinity for sodium and potassium ions. In comparison calcium and magnesium were retained even more tenaciously. The possible utility of aqueous alcohol solutions in other chromatographic procedures using ion exchange resins is suggested again by these results. Hirs, Moore, and Stein have reported the value of ethyl alcohol–buffer mixtures in the separation of several amino acids with similar chromatographic characteristics (2).

The application of this procedure to 4% perchloric acid extracts of whole blood, kidney, and liver was tested. After approximately 80% neutralization of the perchloric acid with potassium hydroxide and removal of the precipitated potassium perchlorate, the samples were desalted as described. Excellent chromatographic resolution of the amino acids in the ethyl alcohol–hydrochloric acid eluate derived from 0.5 ml. of blood or 100 mg. of fresh liver and kidney was obtained using the pH 12 buffered–phenol solvent system of McFarren (4).

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## Improved Purification of Tetramethylammonium Chloride for Polarographic Studies

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THE high negative discharge potential of tetramethylammonium chloride makes it useful as a supporting electrolyte for polarographic studies. In addition, its solubility in alcohol enhances its value in work on many water-insoluble organic compounds. The technical grade quaternary salt is contaminated and frequently shows several reduction waves before the discharge potential of the tetramethylammonium ion. The usual purification method is recrystallization from ethyl alcohol— as many as four recrystallizations frequently being necessary— and is accompanied by high loss of the product.

A superior recrystallization medium has been found to be methanol in acetone. About 10 grams of the salt is dissolved in 100 ml. of hot 25% methanol in acetone. The hot solution is filtered, 100 to 110 ml. of acetone is added, and the solution is allowed to cool. The crystalline material is filtered and dried in a vacuum desiccator. The yield of salt of sufficient purity for use as a supporting electrolyte in aqueous or alcoholic media is 60 to 65%.